Internalizing Mental Disorders in HIV: The Role of Environment, Telomere Length and Selected Genetic Variants

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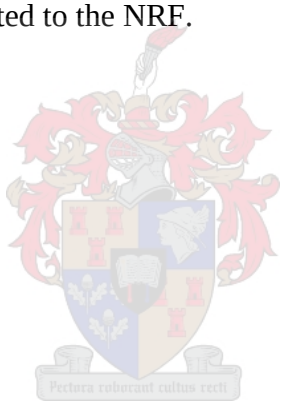

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DECLARATION

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Date: 26/02/2020

This dissertation includes one original paper published in a peer reviewed journal and two unpublished manuscripts. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

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ABSTRACT

Background: HIV+ children and adolescents (CA-HIV) suffer from a considerable burden of internalizing mental disorders (IMDs), which are associated with negative outcomes, such as poor academic functioning and faster HIV disease progression. IMDs are complex disorders with unknown etiology, despite significant research efforts. This PhD study aimed to investigate whether acute stress interacts with vulnerability factors (genetic, acquired or a combination of both) to influence the occurrence of IMDs.

Methods: The study used data from 736 Ugandan CA-HIV who were recruited into a larger study examining mental health among children and adolescents living with HIV/AIDS in Kampala and Masaka districts of Uganda (CHAKA Study). Cases (n = 368) were CA-HIV who had any internalizing mental disorder (IMD) (depressive disorders, anxiety disorders and PTSD). Controls (n = 368) were CA-HIV who did not have these disorders and were matched with cases on age, site, socio-economic status and sex. Psychiatric disorders were assessed using the Child and Adolescent Symptom Inventory-5 (CASI-5) and the Youth Inventory-4R. Chronic and acute stress classes were determined by hierarchical cluster analysis using an index derived from data on social disadvantage variables. DNA extracted from blood was used for targeted genetic investigations. Specifically, variants in the serotonin transporter gene (*SLC6A4: 5-HTTLPR*, rs25531, 5-*HTTLPR*/rs25531 and *STin2* VNTR), and tryptophan hydroxylase gene (*TPH2:* (rs1843809, rs1386494, rs4570625 and rs345177220) were determined. Relative telomere length (TL) was also determined using these samples.

Statistical analysis: Socio-demographic variables were compared between cases and controls. Linear regression was used to determine the association between TL and IMDs. Logistic regression was used to: i) determine the association between acute stress and IMDs, and ii) assess the moderating effect of each of the vulnerability factors on the associations identified in (i).

Results: Presence of IMDs was associated with accelerated TL attrition over a 12-month period. The *T*-allele of *TERT* rs2736100 and the *C*-allele of *TERC* rs16847897 were associated with accelerated TL attrition among cases of IMDs (p = 0.007 and p = 0.012, respectively). CA-HIV who experienced severe acute stress were twice as likely to have an IMD compared to CA-HIV who experienced mild acute stress (p = 0.001). Acute stress significantly interacted with chronic stress (p = 0.033) and *5-HTTLPR*/rs25531 haplotype (p = 0.049) to influence the likelihood of having an IMD. CA-HIV who experienced severe acute stress and severe chronic stress were four times more likely to be a case of IMDs as compared to those under mild acute stress and mild chronic stress. CA-HIV who possessed the *S-A-S-A* haplotype and experienced moderate or severe acute stress were respectively fifteen and twelve times more likely to be diagnosed with an IMD compared to CA-HIV who possessed the *L-A-L-A* haplotype and experienced mild acute stress.

Conclusions: IMDs were associated with accelerated TL attrition. Severe chronic stress and *5-HTTLPR*/rs25531 haplotype independently interacted with acute stress to increase risk for IMDs among Ugandan CA-HIV. These data support previously identified relationships between IMDs and accelerated biological aging, and provide evidence for the role of serotonin gene-environment interactions in the risk of developing IMDs among CA-HIV in Uganda.

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OPSOMMING

Agtergrond: MIV+ kinders asook adolessente (KA-MIV) lei aan hoë vlakke van internaliserende geestesversteurings (IGVs), wat geassosieer word met negatiewe uitkomstes soos swak akademiese prestasie en vinniger MIV vordering. IGVs is ‘n komplekse groep toestande, met ‘n onbekende etiologie, ten spyte van onlangse vordering in die navorsing. In hierdie PhD studie ondersoek ons of akute stres kan kombineer met ander kwesbaarheid faktore (geneties, verworwe of beide) om na ‘n verhoogde voorkoms van IGVs te lei.

Metode: Die huidige studie vorm deel van ‘n groter projek wat geestesgesondheid in 736 kinders en adolessente met MIV/VIGS in Kampala en Masaka distrikte in Uganda (CHAKA studie) ondersoek. Gevalle (n=368) is positief vir KA-MIV en ly aan ‘n IGV (depressie, angs- en/of post-traumatiese stres versteurings). Kontroles is vry van geestesversteurings met vergelykbare ouderdom, perseel, sosio-ekonomiese status en geslag. Psigiatriese toestande was ondersoek met die “Child and Adolescent Symptom Inventory-5 (CASI-5)” en die “Youth Inventory-4R”. Beide kroniese en onlangse stres groepe was bepaal deur ‘n hiërargiese groepontleding. Sosiale benadeling veranderlikes was gebruik as ‘n indeks in die analise. Bloed DNS was gebruik in gerigte genetiese analises. Meer spesifiek, variante in die serotonienvervoerder geen (*SLC6A4: 5-HTTLPR*, rs25531, 5-*HTTLPR*/rs25531 en *STin2* VNTR), en tryptofaan-hidroksilase geen (*TPH2:* (rs1843809, rs1386494, rs4570625 en rs345177220) was bepaal. Relatiewe telomeer lengte (TL) was ook bepaal met dieselfde bloedmonsters.

Statistiese analise: Sosio-demografiese veranderlikes was vergelyk tussen gevalle en kontroles. ‘n Lineêre regressie analise was gebruik om die assosiasie tussen TL en IGVs te bepaal. ‘n Logistieke regressie model was gebruik om: i) die assosiasie tussen onlangse stres en IGVs te bepaal, en ii) die modererende effek van elk van die kwesbaarheid faktore op assosiasies wat geïdentifiseer was in (i).

Resultate: Die teenwoordigheid van IGVs was geassosieer met ‘n versnelde TL-verkorting oor ‘n 12 maande periode. Die *T*-aliel van *TERT* rs2736100 en die *C*-aliel van *TERC* rs16847897 was geassosieer met ‘n versnelde TL-verkorting in IGV gevalle ($p = 0.007$ en $p = 0.012$, onderskeidelik). KA-MIV wat erge akute stress ondervind het was twee keer meer geneig om ‘n IGV te hê in vergelyking met KA-MIV wat ‘n ligte akute stres ervaring gehad het ($p = 0.001$). Akute stres het ‘n beduidende interaksie gehad met kroniese stres ($p = 0.033$) en *5-HTTLPR*/rs25531 haplotipe ($p = 0.049$) wat bepaal het hoe waarskynlik die teenwoordigheid van ‘n IGV was. KA-MIV wie erge akute en kroniese stres ervaar het was vier keer meer geneig om ‘n IGV geval te gewees het, in vergelyking met ligte akute stres asook ligte kroniese stres. KA-MIV wie ‘n *S-A-S-A* haplotipe gehad het en wie matige of erge stres ervaar het was onderskeidelik vyftien en twaalf keer meer geneig om ‘n diagnose van ‘n IGV te gehad het, in vergelyking met KA-MIV wie ‘n *L-A-L-A* haplotipe en ligte akute stres ervaar het.

Slotsom: IGVs was geassosieer met ‘n versnelde TL verkorting. Erge kroniese stres en *5-HTTLPR*/rs25531 haplotipe toon ‘n onafhanklike interaksie met akute stres en verhoogte risiko vir IGVs onder KA-MIV van Uganda. Die data ondersteun ‘n verhouding tussen IGVs en versnelde biologiese veroudering wat al van te vore geïdentifiseer is, en verskaf bewyse vir ‘n interaksie tussen serotonien geen en die omgewing in die kwesbaarheid vir IGVs onder KA-MIV in Uganda.

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Nature of contribution Extent of contribution (%)

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The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to chapters 2, 3 and 4 *in the dissertation*,

2. No other authors contributed to chapters 2, 3 and 4 besides those specified above, and

3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made for submitting the manuscript in chapter 4 of this dissertation to a peer-reviewed journal.

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DEDICATION

To my loving wife Betty Nassimbwa, for all the patience, encouragement and tremendous support. My Children: Angella Dianah Naggayi Kalungi, Mark Kibirige Kalungi & Ariana Martha Kalungi for always respecting my reading.

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CHAPTER ONE
INTRODUCTION

1.1 Discussion of the central research theme

Internalizing mental disorders (IMDs) (namely, depressive disorders, anxiety disorders and post-traumatic stress disorder (PTSD)) are leading causes of disability worldwide (World Health Organization, 2017; Akena *et al*., 2012; Murray *et al*., 2012). IMDs often develop in childhood and adolescence, and, if left untreated, can go on into adulthood and negatively impact psychosocial function and add to the healthcare burden (Jenkins *et al*., 2011). As such, early diagnosis and intervention are vital.

The human immunodeficiency virus/acquired immunodeficiency disease syndrome (HIV/AIDS) is a significant global health burden, with approximately 37.9 million people living with the virus globally (UNAIDS, 2019). With an estimated 1.85 million infected, eastern and southern Africa accounts for more than 60% of children and adolescents living with HIV globally (UNAIDS, 2018). In Uganda, the country with the fifth-highest HIV prevalence in the region, an estimated 140,000 children and adolescents are living with the virus (UNAIDS, 2018).

HIV-positive (HIV+) children and adolescents suffer a considerable burden of IMDs (Kinyanda *et al*., 2019; Mellins *et al*., 2012; Nachman *et al*., 2012). Studies undertaken both in the developed world (Europe and the United States) and in sub-Saharan Africa have documented rates of depression of between 12.7 and 40% (Lwidiko *et al*., 2018; Kim *et al*., 2014; Kamau *et al*., 2012; Gadow *et al*., 2012; Mellins *et al*., 2012; Nachman *et al*., 2012; Musisi & Kinyanda, 2009) and rates of anxiety disorders of 9 to 32.2% (Kinyanda *et al*., 2019; Kamau *et al*., 2012; Mellins *et al*., 2012; Nachman *et al*., 2012) among HIV+ children and adolescents. These estimates are far greater than the estimated worldwide prevalence of 2.6% and 6.5% for any depressive and anxiety disorder, respectively, among children and adolescents (Polanczyk *et al*., 2015). For IMDs alone, rates of 12% and 27% among HIV+ children and adolescents have been documented in Uganda and South Africa (Kinyanda *et al*., 2019; Woollet, Cluver, Bandeira & Brahmbatt, 2017). Among persons living with HIV/AIDS (PLHIV), IMDs have been associated with a number of negative outcomes, including more rapid HIV disease progression (Chida & Vedhara, 2009; Ironson *et al*., 2005), poor adherence to medication (Kinyanda *et al*., 2018; Springer, Dushaj & Azar, 2012), risky sexual behavior

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(Kinyanda *et al*., 2018; Springer, Dushaj & Azar, 2012), poor linkage to care for newly diagnosed HIV+ persons *(*Bhatia *et al*., 2011*)*, increased HIV transmission (through the promotion of HIV drug resistance) (Remien & Mellins, 2007) and impaired academic and social functioning (Nachman *et al*., 2012).

IMDs are multifactorial disorders with complex etiology, which is increasingly recognized as having both biological and psychosocial contributions. The role of stressful environments and the physiology of the stress response system have been broadly implicated in the pathophysiology of psychiatric disorders and have been most closely linked to three IMDs (depressive and anxiety disorders, and PTSD) (Smoller *et al*., 2016). Psychosocial stress represents the most prevalent environmental risk factor for IMDs (Popoli *et al*., 2012). The diathesis-stress hypothesis of neuropsychiatric disorders postulates that a lower stress threshold is required for psychiatric disease to occur in individuals who harbor certain vulnerability factors, which may be genetic and/or acquired (Caspi *et al*., 2003; Silberg *et al*., 2001; Monroe & Simons, 1991). However, the diatheses that lead to increased susceptibility to the harmful effects of psychosocial stress remain largely unknown. Exposure to chronic stress has been associated with an increased risk for IMDs (Adelman *et al*., 2014; Evans, Li, & Whipple, 2013; Revenson *et al*., 2016; Robles, Slatcher, Trombello, & McGinn, 2014; Charney & Manji, 2004; de Kloet *et al*., 2005). HIV+ children and adolescents have been reported to experience various chronic life stressors such as knowledge of their HIV-status, increased levels of stigma and poor parental mental health (Betancourt *et al*., 2014). These life stressors may thus exacerbate the occurrence of IMDs among HIV+ children and adolescents.

Genetic factors have been found to contribute to the etiology of IMDs. Twin studies have estimated a genetic heritability of 35% for depression (Otte *et al*., 2016) and 30-50% for PTSD (Smoller *et al*., 2016), while a meta-analysis of family and twin studies estimated a genetic heritability of 10−48% for anxiety disorders among participants with unknown HIV status (Hettema *et al*., 2005; Hettema, Neale & Kendler, 2001). Despite this high heritability, the underlying etiology, biochemical or molecular events leading to IMDs are largely unknown (Sullivan *et al*., 2017; Levinson *et al*., 2014). Serotonergic neurotransmission has, however, been implicated in the etiology of IMDs due to the beneficial effects of selective serotonin re-uptake inhibitors (SSRIs) in the treatment of IMDs (Jakubovski *et al*., 2019; Ernst, Mechawar, & Turecki, 2009). Genes encoding components of the serotonergic neurotransmission pathway are therefore ideal candidates to investigate in IMDs. Indeed, the serotonin transporter gene

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(solute carrier family 6 member A4, *SLC6A4*) has been implicated in the development of IMDs (Arango *et al*., 2001; Malison *et al*., 1998), and polymorphisms within the human tryptophan hydroxylase 2 gene (*TPH2*), which is involved in serotonin synthesis, have also been associated with IMDs and have been reported to influence antidepressant response to SSRIs (Gassó *et al*., 2017).

Chronic stress exerts broad effects on physiology, which could act as objective markers of stress exposure or alternatively contribute to the pathoetiology of mental disorders. Chronic stress has been reported to increase oxidative stress in the central nervous system (CNS) (Schiavone *et al*., 2013), and prooxidative environments have, in turn, been associated with telomere shortening (Wolkowitz *et al*., 2011a). Telomeres are deoxyribonucleic acid (DNA) repeat structures at the ends of chromosomes that serve to protect chromosomes from fusing together during mitosis (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006). Telomeres shorten at the end of each mitotic cycle, due to the inability of the DNA polymerase enzyme to fully replicate the 3' end of the DNA strand, a process termed the "end replication problem" (Watson, 1972). Exposure to chronic stress has been associated with shorter telomeres (Damjanovic *et al*., 2007; Epel *et al*., 2004; Notterman & Mitchell, 2015; Parks *et al*., 2009; Shalev, 2012; Shalev *et al*., 2013). Increased maternal adverse childhood experiences have been reported to associate with shorter infant telomere length (TL), and TL attrition in infancy has been reported to interact with maternal adverse childhood experiences to predict higher problematic externalizing behaviors (Esteves *et al*., 2019). Shorter TL has also been associated with IMDs among children and adolescents (Verhoeven *et al*., 2015; Verhoeven *et al*., 2014; O'Donovan *et al*., 2011), and has been described as a risk factor for IMDs by some studies (Shalev *et al*., 2014; Gotlib *et al*., 2015) and a marker of increased stress exposure (Needham *et al*., 2015; Zhang *et al*., 2014; Houben, Moonen, van Schooten, & Hageman, 2008). There is thus a need to understand whether accelerated telomere shortening is causally linked to IMDs, or whether the development of IMDs and the shortening of telomeres are simultaneous effects of increased stress exposure. In addition to environmental exposures, TL is also at least partially genetically determined. Therefore, investigations into the role of telomeres in IMDs should take genetic variants in loci implicated in TL maintenance into account (Codd *et al*., 2013).

Biological psychiatry studies investigating the molecular etiology of IMDs have been conducted in predominantly European and North American white populations. However, these findings are not necessarily relevant to populations of African ancestry, who have very high

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levels of genetic diversity (Tishkoff *et al*., 2009; Ingman, Kaessmann, Pääbo & Gyllensten, 2000). For this reason, studies investigating genetic risk for IMDs, as well as how genetic factors influence susceptibility to the harmful effects of stress need to be conducted in African populations.

The present study utilizes the diathesis-stress model to investigate genetic and environmental risk for IMDs in an understudied, at-risk population i.e. HIV+ Ugandan children and adolescents. The following sections of the introduction will more closely examine the available literature on genetic and environmental determinants of IMDs and provide a rationale for the study approach employed.

1.2 Background literature

1.2.1 Occurrence of psychiatric disorders among HIV+ children and adolescents

HIV/AIDS in children and adolescents is associated with both medical and psychiatric morbidity, in addition to delayed motor and cognitive development (Van Rie, Harrington, Dow, & Robertson, 2007). HIV+ children and adolescents experience high rates of psychiatric disorders, with estimates ranging from 17.4 – 61% (Kinyanda *et al*., 2019; Gadow *et al*., 2012; Gadow *et al*., 2010; Malee *et al*., 2011; Mellins *et al*., 2012; Musisi & Kinyanda, 2009; Pine *et al*., 1998; Reinherz *et al*., 2003), which is substantially higher than the worldwide pooled prevalence of 13.4% for any psychiatric disorder among children and adolescents (Polanczyk *et al*., 2015). Among a general population of children and adolescents, psychiatric disorders are associated with psychological distress and impaired quality of life (Weitkamp, Daniels, Romer & Wiegand-Grefe, 2013). Among HIV+ children and adolescents, psychiatric disorders are further associated with negative clinical and behavioral outcomes, non-adherence to anti-retroviral treatment (Malee *et al*., 2011; Walkup *et al*., 2009), and increased stigma and isolation (Betancourt *et al*., 2014).

Internalizing mental disorders (IMDs) are the most commonly reported psychiatric disorders among HIV+ children and adolescents (Kinyanda *et al*., 2019; Gadow *et al*., 2012; Gadow *et al*., 2010; Malee *et al*., 2011; Mellins *et al*., 2012; Musisi & Kinyanda, 2009), and were thus selected for further investigation in this study.

1.2.2 Internalizing mental disorders: general classification and diagnosis

Internalizing mental disorders are characterized by quiet, internal distress (Tandon, Si, & Luby, 2011). Children who suffer from IMDs usually internalize their problems, in contrast to

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children with externalizing disorders, who display overtly socially negative or disruptive behavior (Tandon, Si & Luby, 2011). IMDs are associated with high levels of negative emotions (Regier, Kuhl, & Kupfer, 2013; Turygin, Matson, Beighley, & Adams, 2013), which include anger, contempt, disgust, guilt and fear (Koch, Forgas & Matovic, 2013). IMDs comprise a broad range of disorders, which have been classified as depressive or anxiety disorders, and PTSD.

1.2.2.1 Depression

Depression is characterized by persistent low mood, loss of interest in formerly pleasurable activities, feelings of worthlessness and hopelessness, suicidality, reduced psychomotor tone, poor concentration, changes in appetite and poor sleep (American Psychiatric Association, 2013). Insomnia, excessive sleeping, fatigue, aches, pains, digestive problems and/or reduced energy may also be present (National Institute of Mental Health, 2019). Diagnosis of depression requires the presence of both core and associated symptoms. Core symptoms include sadness, unhappiness or irritability, and anhedonia, while associated symptoms include negative thinking, lack of energy, difficulty concentrating, and disturbances in sleep and appetite (American Psychiatric Association, 2013; Rey, Bella-Awusah & Liu, 2015). These symptoms must be present daily, for a duration of at least two weeks (American Psychiatric Association, 2013), must result in impaired functioning or significant subjective distress, and should not be a manifestation of the effects of a substance or an underlying medical condition (American Psychiatric Association, 2013; Rey, Bella-Awusah & Liu, 2015). Among children (aged 5-11 years), depression presents with irritability (temper tantrums, noncompliance), reactive affect, somatic complaints and is frequently comorbid with anxiety, behavior problems, and attention-deficit/hyperactivity disorder (ADHD).

Among adolescents (aged 12-17 years), depression presents with irritability (grumpiness, hostility, frustration and outbursts of anger), reactive affect, hypersomnia, increased appetite and weight gain, somatic complaints, and extreme sensitivity to rejection (Rey, Bella-Awusah & Liu, 2015). In adults, it presents with anhedonia, psychomotor agitation, diurnal variation, lack of affective reactivity and early morning waking (Rey, Bella-Awusah & Liu, 2015).

Depression is the leading cause of disability worldwide (World Health Organization, 2017) and accounts for 40.5% of disability-adjusted life years (Whiteford *et al*., 2013). Depression is common among PLHIV and provides particular challenges to clinical care of PLHIV,

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impacting on adherence to treatment, viral suppression, self-care and quality of life (Mutumba *et al*., 2017; Simoni *et al*., 2011). Prevalence rates of between 12.7 to 63% have been reported among HIV+ children and adolescents in various regions of the world (18.9% in Malawi (Kim *et al*., 2014), 17.8% in Kenya (Kamau *et al*., 2012), 18.5% in Malaysia (Rosliwati *et al*., 2008), 35.5% in Ethiopia (Abebe *et al*., 2019), 40% in Uganda (Musisi & Kinyanda, 2009), 63% in Zimbabwe (Mavhu *et al*., 2013) and 12.7 -52% in the United States (Mellins & Malee, 2013; Mellins *et al*., 2012; Nachman *et al*., 2012)). The prevalence estimate observed in Uganda is almost double the estimated lifetime estimate of 23.2% for depression that was observed in the general population in the United States (Heo *et al*., 2008).

Risk factors for depression among children and adolescents include, among others, a family history of depression, parental mental disorder, pubertal stage, childhood maltreatment, parental substance use or alcohol misuse, parental loss, medical illness, social rejection, chronic pain, parent-child conflict, female gender, previous history of depression, and bullying and trauma (Rey, Bella-Awusah & Liu, 2015; Lindert *et al*., 2014; Gardner, Thomas & Erskine 2019; Sheng *et al*., 2017; Davey, Yücel, & Allen, 2008). Genetic factors have also been described as risk factors for depression in both adults and children (Sullivan, Neale MC & Kendler, 2000). An in-depth discussion of genetic mechanisms in depression is provided in Section 1.2.3. There are several assessment tools for measuring depression among children and adolescents. Tools that have been commonly used are summarized in Table 1.1 below.

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Table 1.1: Assessment tools for depression among children and adolescents

Assessment tool (Reference)	Description	Validation/Local adaptation among CA-HIV in Uganda
The Child and Adolescent Symptom Inventory-Edition 5 (CASI-5)[f] (Gadow & Sprafkin, 2013)	The CASI is an assessment tool for DSM-5 defined psychiatric disorders in youth. It has the advantage of having a symptom category that assesses both social and academic functioning of the child and is suitable for children aged 5 to 18 years. It was deemed to be an ideal tool for the present study as it includes an assessment of impairment and is suited for the age category of participants in the present study (5-17 years). The CASI-5 has the disadvantage of lacking both scoring software and a computer-presented version.	Locally adapted (Kinyanda *et al*., 2019; Mpango *et al*., 2017)
The youth's inventory-4 (YI-4R)[f] (Gadow & Sprafkin, 199)	The YI-4R is an assessment tool for DSM-4 defined psychiatric disorders in youth. It has the advantage of having a symptom category that assesses both social and academic functioning and is suitable for youths aged 12 to 18 years. The YI-4R has a disadvantage of lacking both scoring software and a computer-presented version.	Locally adapted (Kinyanda *et al*., 2019: Mpango *et al*., 2017)
The Center for Epidemiologic Studies-Depression scale for children (CES-DC) (Faulstich *et al*., 1986)	The CES-DC is a 20-item self-report depression inventory with possible scores ranging from 0 to 60. The scale has good psychometric properties in adolescents despite its poor reliability and validity in younger children. The CES-DC was originally validated in a sample of 8-17 year old children in the United States (78% boys, 55% white, 39% black and 4% other).	Neither validated nor locally adapted
The Mood and Feelings Questionnaire (MFQ) (Angold *et al*., 1995)	The MFQ has 26 items on the short form version and 66 items on the long form version, and is suitable for children aged 6 to 17 years. The MFQ was first validated in a sample of children in the United States.	Neither validated nor locally adapted
The Depression Self-Rating Scale (DRSS) (Birleson, 1981)	The DRSS is a brief, simple to use self-report scale that takes a few minutes for a child to complete. This tool is suitable for children aged 8 to 14 years.	Neither validated nor locally adapted

[f]Used in the present study to assess for depression, CA-HIV = HIV+ children and adolescents.

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1.2.2.2 Anxiety disorders

Anxiety disorders are characterized by significant feelings of anxiety and fear (American Psychiatric Association, 2013). Anxiety refers to a worry about the future, while fear is a reaction to current events (American Psychiatric Association, 2013). The average age of onset of anxiety disorders has been reported to be 21.3 years (de Lijster *et al*., 2017), but varies according to the specific type of disorder (Table 1.2).

Table 1.2: Average age of onset for each anxiety disorder

Anxiety disorder	Age of onset	Reference
Separation anxiety disorder	7 – 8 years	Rapee, 2018; American Psychiatric Association, 2013
Generalized anxiety disorder	Mid-adolescence to adulthood	Rapee, 2018; American Psychiatric Association, 2013
Social phobia	11 – 13 years	Rapee, 2018; American Psychiatric Association, 2013
Specific phobias	6 -7 years	Rapee, 2018; American Psychiatric Association, 2013
Panic disorder	20 – 24 years	Rapee, 2018; American Psychiatric Association, 2013
Agoraphobia	21 – 35 years	de Lijster *et al*., 2017; American Psychiatric Association, 2013

Anxiety disorders impact heavily on physical, social, occupational and educational health, and account for 14.6% of disability-adjusted life years in a general population (Whiteford *et al*., 2013). Prevalence rates of 9 to 58.5% for these disorders have been reported among HIV+ children and adolescents in different studies the United States and East Africa (Kinyanda *et al*, 2019; Mellins *et al*., 2012; Nachman *et al*., 2012; Kamau *et al*., 2012; Musisi & Kinyanda,

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2003). In Uganda, a prevalence of 58.5% for anxiety disorders was reported among HIV+ adolescents (n = 82) (Musisi & Kinyanda, 2003). This is considerably higher than the prevalence rates of 16.7% and 12% that were reported among HIV-negative school-going orphans and non-orphans (10–13 years) (n = 420), respectively, in Rakai, a rural area of Uganda (Musisi, Kinyanda, Nakasujja & Nakigudde, 2007), and the 26.6% prevalence rate that was reported among a community sample of children and adolescents (n = 1,680) from war affected areas in north-eastern Uganda (Abbo *et al*., 2013).

Risk factors for anxiety disorders include family history, life events, cognitive biases, genetic and temperamental factors (Rapee, 2018). The core features for each specific anxiety disorder are summarized in Table 1.3, below.

Table 1.3: Core features of specific anxiety disorders

Anxiety disorder	**Core features**
Separation anxiety disorder	Fear or concern that something bad will happen to the child or attachment figure (commonly a parent) when they are separated and as a result of this belief, the child avoids separation from the attachment figure.
Generalized anxiety disorder	Tendency to worry about a wide range of negative possibilities, that something bad will happen.
Social anxiety disorder	Fear and avoidance of social interactions or social performance due to a belief that others will negatively evaluate the child.
Specific phobias	Fear and avoidance in response to a range of specific cues, situations, or objects. There is a common belief that the object or situation will lead to personal harm.
Panic disorder	Experience and fear of unexpected panic attacks, commonly involving several somatic symptoms and fears of dying or going crazy.
Agoraphobia	Additional fear and avoidance of several "agoraphobic" situations, commonly due to a fear of experiencing a panic attack in those situations.

Reference: Rapee, 2018.

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A number of assessment tools for anxiety disorders among children and adolescents have been developed and are summarized in Table 1.4.

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Table 1.4: Assessment tools for anxiety disorders among children and adolescents

Assessment Tool (Reference)	Description	Validation/ Local adaptation among CA-HIV in Uganda
The Child and Adolescent Symptom Inventory-Edition 5 (CASI-5)[f] (Gadow & Sprafkin, 2013)	Please see Table 1.1	Locally adapted (Kinyanda *et al*., 2019; Mpango *et al*., 2017)
The Youth's Inventory-4 (YI-4)[f] (Gadow & Sprafkin, 1999)	Please see Table 1.1	Locally adapted (Kinyanda *et al*., 2019: Mpango *et al*., 2017)
The Spence Children's Anxiety Scale (SCAS) (http://www.scaswebsite.com/)	The SCAS assesses symptoms of various anxiety disorders using dimensions proposed by DSM-4. This tool assesses 44 items in six domains of anxiety and is suitable for young people aged between 8-15 years.	Neither validated nor locally adapted
Screen for Anxiety and Related Disorders (SCARED) (http://www.pediatricbipolar.pitt.edu/resources/instruments)	The SCARED is a child and parent self-report instrument for screening various childhood anxiety disorders which consists of 41 items and five factors that parallel the DSM-IV classification of anxiety disorders. The SCARED has good internal test- retest reliability, discriminant validity and sensitivity to treatment response.	Neither validated nor locally adapted
The Multidimensional Anxiety Scale for Children. 2nd Edition–Self-Report (MASC 2™–SR) (https://www.mhs.com/MHS-Assessment?prodname=masc2)	The MASC 2™–SR is a comprehensive assessment of anxiety dimensions in children and adolescents aged 8 to 19 years. It indexes the range and severity of anxiety symptoms, and is a useful tool for early identification of anxiety-prone youth, as well as in monitoring treatment effects.	Neither validated nor locally adapted
The Preschool Anxiety Scale, Revised (PASR) (https://www.mq.edu.au/research/research-centres-groups-and-facilities/healthy-people/centres/centre-for-emotional-health-ceh/resources)	The PASR was adopted from the SCAS and is the only measure that specifically assesses a wide range of anxiety symptoms in preschool-aged children and utilizes parent report. It is designed to assess symptoms of anxiety and fears in children aged 6 years and below.	Neither validated nor locally adapted
The Revised Children's Manifest Anxiety Scale (RCMAS) (Reynolds & Richmond, 1985)	The RCMAS is a 37-item, self-report instrument with only "yes" and "no" options and is designed to assess the level and	Neither validated nor locally adapted

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	nature of anxiety among children between 6 and 9 years of age. It has a disadvantage of inconsistency of the reliability scale.	
The State Trait Anxiety Inventory for Children (STAIC) (http://www.mindgarden.com/products/staisch.htm)	The STAIC comprises of two 20-item scales for measuring state and trait anxiety in children between the ages of 8 and 14 years.	Neither validated nor locally adapted
The Beck Anxiety Inventory for Youth (BAI-Y) (https://www.pearsonclinical.com/psychology/products/100000153/beck-youth-inventories-second-edition-byi-ii.html)	The BAI-Y is a 21-item multiple-choice self-report inventory that measures the severity of an anxiety in adults and adolescents. It has an advantage of clearly discriminating anxiety from depression.	Neither validated nor locally adapted
The Fear Survey Schedule for Children Revised (FSSCR) (http://onlinelibrary.wiley.com/doi/10.1002/9780470713334.app3/pdf)	The FSSC-R is a widely used self-report questionnaire that purports to measure the number of fears and the overall level of fearfulness in children.	Neither validated nor locally adapted
The Social Phobia and Anxiety Inventory for Children (SPAIC) (Beidel *et al*., 2000)	The SPAIC is a 26-item tool that evaluates the somatic, cognitive, and behavioral aspects of Social Phobia and anxiety in children between the ages of 8 and 14.	Neither validated nor locally adapted
The Children's Anxiety Sensitivity Index (CASI) (Silverman *et al*., 1991)	The CASI has an 18-item scale and assesses for anxiety sensitivity by asking children to state how aversively they view anxiety symptoms.	Neither validated nor locally adapted

[f]Used in the present study.

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Anxiety disorders in childhood and adolescence have been associated with impaired quality of life, poor academic performance and recurrence later in life (Nachman *et al*., 2012; Ezpeleta *et al*., 2001; Mendlowicz & Stein, 2000; Woodward & Fergusson, 2001), as well as increased risk of suicide attempts and suicidal ideation (Ortin *et al*., 2019; Herres *et al*., 2019). Anxiety disorders also affect somatic health, and have been associated with irritable bowel syndrome, cardiovascular disease and chronic pain (Roy-Byrne *et al*., 2008). Among adults, anxiety disorders have been associated with loss of worker productivity (Kessler & Frank, 1997), elevated rates of general medical disorders (Zaubler & Katon, 1996), frequent comorbid mental disorders (Compton, Thomas, Stinson, & Grant, 2007; Conway, Compton, Stinson, & Grant, 2006; Kessler & Frank, 1997), increased risk of suicide attempts and suicidal ideation (Pilowsky *et al*., 2006), and high mortality rates (Pratt, Druss, Manderscheid & Walker, 2016).

1.2.2.3 Posttraumatic stress disorder (PTSD)

Posttraumatic stress disorder can develop after exposure to a traumatic event, such as sexual assault, warfare, or traffic collisions (Bisson, Cosgrove, Lewis, & Roberts, 2015; American Psychiatric Association, 2013). Symptoms of PTSD may include disturbing thoughts, feelings, or dreams related to the events, mental or physical distress to trauma-related cues, attempts to avoid trauma-related cues, alterations in how a person thinks and feels, and an increase in the fight-or-flight response (American Psychiatric Association, 2013). A diagnosis of PTSD requires: i) one symptom of intrusion including recurrent distressing dreams, recurrent distressing memories of the trauma, dissociative reactions (e.g., flashbacks), or intense psychological or physiological reactivity to reminders of the trauma; ii) persistent avoidance of internal or external stimuli associated with the trauma; iii) two symptoms of negative alterations in cognitions and mood associated with the trauma including persistent, distorted blame of self or others, persistent negative emotional state (e.g., fear, horror, anger, shame or guilt), diminished interest or participation in significant activities, detachment or estrangement from others, or persistent inability to experience positive emotions (e.g., emotional numbing); and iv) two symptoms of alterations in arousal and reactivity, including irritability or aggressive behavior, reckless or self-destructive behavior, hypervigilance, exaggerated startle responding, problems with concentration, or sleep disturbance. The diagnosis requires that symptoms last at least one month, are associated with clinically significant distress or impairment, and are not associated with the effects of a substance or an underlying medical condition (American Psychiatric Association, 2013; Bui *et al*., 2014). Posttraumatic stress disorder negatively impacts on a spectrum of functioning, having been associated with,

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elevated rates of age-related diseases (Šagud *et al*., 2017), frequent comorbid mental disorders (Mason, LeBouthillier & Asmundson, 2019), impaired physical functioning (Hall *et al*., 2014), and higher mortality (Boscarino, 2006).

The lifetime prevalence of PTSD among the general population has been found to be approximately 7% in the United States (Kessler and Wang, 2008). Systematic reviews have documented prevalence rates of 20% among survivors of road traffic accidents in Europe, the United States and China (Dai *et al*, 2018), 23.7% among earthquake survivors (Bassuk, Richard & Tsertsvadze, 2015) and 47% among children exposed to war (Attanayake *et al*., 2009). In a representative population sample of 10-13 year old children followed up for at least 3 years in the United States, approximately 30% had experienced one or more traumatic events by age 16, and 13% endorsed symptoms of PTSD (Copeland *et al*., 2007). From these data, it is apparent that not all individuals exposed to trauma develop the disorder and thus individual factors, including genetic background and environment, must influence risk and resilience to the harmful effects of trauma (Smoller *et al*., 2016).

Numerous studies have investigated such differential vulnerability to PTSD. This research has indicated a role for genetic factors (Nievergelt *et al*., 2019) (discussed in Section 1.2.3), as well as individual differences and features specific to the trauma exposure. More specifically, exposure to trauma such as a natural disaster, war or a motor vehicle crash (Trickey *et al*., 2012; Attanayake *et al*., 2009), female sex (McLaughlin *et al*., 2013), family characteristics (Morris, Gabert-Quillen & Delahanty 2012) and cognitive and emotional responses to the traumatic event (Jenness *et al*., 2016; Trickey *et al*., 2012; Ehlers, Mayou, & Bryant 2003) have been identified as increasing the risk of developing PTSD. A meta-analysis of 64 studies that assessed risk factors for PTSD among children and adolescents predominantly from Europe and the United States revealed that factors relating to the subjective experience of the traumatic event (including peri-trauma fear and perceived life threat) and post-trauma variables (including low social support, social withdrawal, psychiatric comorbidity, poor family functioning, and the use of certain cognitive strategies such as distraction and thought suppression) accounted for medium-to-large effect sizes in the prediction of PTSD, while pre-trauma factors (including female gender, low intelligence, low socio-economic status (SES), pre-trauma life events, pre-trauma low self-esteem, pre-trauma psychological problems in the youth and parents) accounted for only small-to-medium effect sizes. (Trickey *et al*, 2012). Of relevance to the present study, being diagnosed with HIV can be experienced as traumatic

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among adolescents and young adults (Radcliffe *et al*., 2007) and children orphaned by AIDs have been reported to be 67% more likely to suffer PTSD compared to those orphaned by other causes in South Africa (Cluver, Orkin, Gardner, & Boyes, 2012). Though living with HIV has been reported as a potential source of trauma among children and adolescents, further research on the prevalence of PTSD in this population is required (Ahlers, 2016).

1.2.3 The diathesis-stress hypothesis

The research referenced above provides clear support for the complex nature of psychiatric disorders and indicates that their etiology is likely mediated by a variety of factors producing individual, interactive and multiplicative effects. The diathesis-stress hypothesis of neuropsychiatric disorders postulates that a lower stress threshold is required for psychiatric disease to occur in individuals who harbor certain vulnerability factors, which may be genetic and/or acquired (Caspi *et al*., 2003; Silberg *et al*., 2001; Monroe & Simons, 1991). The hypothesis that inherent differences in vulnerability drive risk for IMDs has informed our approach to the current study and the following sections will examine the evidence for biological factors influencing vulnerability to the development of IMDs.

1.2.4 Heritability of internalizing mental disorders

Findings from numerous studies suggest that genetic predisposition contributes to the etiology of IMDs. Twin studies indicate an estimated genetic heritability of 35% for depression (Otte *et al*., 2016), 30-50% for PTSD (Smoller *et al*., 2016), and 30-67% for anxiety disorders (Domschke & Maron, 2013; Hettema, Neale & Kendler, 2001) among participants with unknown HIV status. Despite these findings, the diagnosis of IMDs is still largely based on clinical symptoms rather than biological markers. This is due to a number of limitations, including the complexity of the underlying pathophysiology; diverse manifestation and course of IMDs; heterogeneity of contributing genetic and non-genetic factors, and limited knowledge about genotype-phenotype relationships (Sullivan *et al*., 2017; Levinson *et al*., 2014). Nevertheless, the increasing number of studies in the field, the development of new approaches that allow for more detailed genetic examinations, and the founding of consortia that can analyze pooled data are greatly increasing the scope and potential of genetic studies.

1.2.4.1 Genome-wide association studies in internalizing mental disorders

Genome-wide association studies (GWASes) powered by large sample sizes allow for the investigation of genetic variants with relatively small effect sizes. An early mega-analysis of depression that included 9,240 cases and 9,519 controls, and replication in a further 6,783 cases

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and 50,695 controls, failed to detect clear genetic variants associated with depression (Ripke *et al*., 2013). However, with an increasing number of studies with sufficiently large sample size, genetic variants associated with depression are being discovered (summarized in Table 1.5). For example, a genome-wide association study (GWAS) by Hyde *et al*. (2016) identified five independent genetic loci associated with risk of major depression in individuals of European descent (75,607 cases and 231,747 controls). Using a single population-based cohort of 322,580 (113,769 cases and 208,811 controls) individuals from the UK Biobank cohort, a GWAS by Howard *et al*. (2018) identified 17 independent loci that significantly associated with depression in the UK Biobank phenotypes and the gene-set enrichment analyses implicated pathways in excitatory neurotransmission, mechanosensory behavior, post-synaptic, neuronal dendritic spine and dendritic function.

A more recent GWASes meta-analysis conducted by the Psychiatric Genetics Consortium Major Depressive Disorder Working Group analyzed seven cohorts (35,458 cases and 344,901 controls of European descent) and identified 44 independent loci that were significantly associated with major depression Wray *et al*. (2018) (see Table 1.5). By demonstrating that the number of significant loci increased as additional cohorts were included in the analysis, this publication highlighted the value of large sample sizes in GWASes of complex disorders such as depression. Interestingly, the loci identified in a previous study of Han Chinese participants, which included 5,303 cases of major depressive disorder and 5,337 controls (Cai *et al*., 2015a), were uncommon in the Wray *et al*. combined seven cohort study group. This finding highlights the fact that genetic findings may not be broadly applicable across populations of different ethnicities, and underscores the value of conducting genetic investigations in diverse populations.

GWASes have also been carried out on anxiety disorders, with early studies suggesting involvement of variants relating to neurotransmitter signaling (Schosser *et al*., 2013) and proprotein convertase 2 (*PCSK2)*, which is involved in neuroendocrine peptide metabolism, for pre-school internalizing problems (Benke *et al*., 2014). A recent GWAS among adults with depression or anxiety (n = 972 for anxiety and n = 832 for depression) and children with anxiety (n = 920) failed to identify single nucleotide polymorphisms (SNPs) that significantly associate with treatment outcome following cognitive behavioral therapy (Rayner *et al*., 2019). In contrast, another recent GWAS (12,655 cases with various anxiety and stress-related diagnoses and 19 225 controls) identified genetic variants located within the phosphodiesterase 4B gene

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(*PDE4B*) to be significantly associated with both anxiety (agoraphobia, GAD, panic disorder, social phobia, specific phobia and other on unspecific anxiety disorder) and anxiety-related disorders (Meier *et al*., 2019). The PDE4B gene has been reported to regulate intracellular cyclic adenosine monophosphate signaling and is strongly expressed in the human brain (Meier *et al*., 2019).

Several recent GWASes have been conducted on PTSD. A recent study by Katrinli *et al*. (2019) that utilized GWAS data on 50 SNPs reported associations between SNPs within the gene for human leucocyte antigen (HLA) and PTSD among samples from an African-American cohort in the United States. In their gene enrichment analysis, Katrinli *et al*. (2019) found the *HLA* variants implicated in PTSD were enriched in pathways relevant to neural and immune activity. Another GWAS by Wang *et al*. (2019) has also implicated perturbation of the inflammatory response in PTSD, with a region near 4q31, close to the interleukin 15 gene, associated with PTSD among a trauma-exposed sample of Danish soldiers deployed to war and conflict zones (n = 2,481). The corticotrophin-releasing hormone receptor 1 gene (*CRHR1*) was also recently associated with genetic risk to intrusive re-experiencing of trauma, the most characteristic symptom cluster of PTSD in a GWAS among adult US veterans of European ancestry (n = 146,660) (Gelernter *et al*., 2019).

Interestingly, there were no significant loci identified for the African American participants included in the study (n = 19,983) (Gelernter *et al*., 2019), highlighting that genetic risk for PTSD may vary across different ethnicities. Finally, similar evidence for ancestry-specific genetic risk was found in a recent GWAS of 30,000 PTSD cases and 170,000 controls (Nievergelt *et al*., 2019). A total of three genome-wide significant loci (two in European (n = 23,000) and one in African (n = 4,000) ancestry analyses) were identified with gene-based analyses implicating genes related to dopamine and immune pathways in PTSD (Nievergelt *et al*., 2019).

Table 1.5: Genome-wide association study findings in internalizing mental disorders

Reference	IMD	Study sample	Genetic loci identified
Hyde *et al*. (2016)	Major depression	75,607 cases and 231,747 controls of European descent	Olfactomedin-4 (*OLFM4*); myocyte enhancer factor 2C (*MEF2C*); transmembrane protein 161B (*TMEM161B*)

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Howard *et al.* (2018)	Depression	113,769 cases and 208,811 controls from the UK Biobank Cohort	Dopamine receptor D2 (*DRD2*); Sortilin Related VPS10 domain containing receptor 3 (*SORCS3*); glutamate metabotropic receptor 5 (*GRM5*); ankyrin repeat and sterile alpha motif domain containing 1B (*ANS1B*); calcium binding protein 1 (*CABP1*); teneurin transmembrane protein 2 (*TENM2*); ephrin type-B receptor 2 (*EPHB2*)
Wray *et al.* (2018)	Major depression	35,458 cases and 344,901 controls using data collected by PGC, UK, Icelandic, United States, and Danish studies	See addendum C
Schosser *et al.* (2013)	Anxiety disorders	1,080 cases with comorbid anxiety and major depression, 422 cases with major depression and 1,588 controls of European descent	Cholecystokinin (*CCK*), dopamine D3 receptor (*DRD3*), gamma-aminobutyric acid receptor gamma 2 (*GABRG2*), adrenergic beta-1 receptor (*ADRB1*), glyoxalase I (*GLO1*), ATP-binding cassette subfamily G member 1 (*ABCG1*)
Benke *et al.* (2014)	Internalizing behaviors	4,596 preschool children of European descent	Proprotein convertase 2 (*PCSK2*)
Rayner *et al.* (2019)	Anxiety and depressive disorder symptom improvement following cognitive behavioral therapy	972 adults and 929 children with anxiety, and 832 adults with depression. All participants were of European descent	No significant variants identified
Meier *et al.* (2019)	Anxiety- and stress-related disorders.	12,655 cases with anxiety- or stress-related disorders and 7,308 controls. All participants were of European descent	Phosphodiesterase 4B gene *(PDE4B).*
Katrinli *et al.* (2019)	PTSD	403 cases and 369 trauma-exposed controls. Participants identified as African American	Human leukocyte antigen *(HLA)*

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Wang *et al.* (2019)	PTSD	462 cases and 2,019 controls of Danish descent	Region 4q31, close to interleukin-15
Gelernter *et al.* (2019)	PTSD	146,660 European American and 19,983 African American US war veterans	Transcription factor 4 (*TCF 4*), KAT8 Regulatory NSL Complex Subunit 1 (*KANSL1*), Serine/Threonine-Protein Kinase (*SRPK2*), mitotic arrest deficient 1 like 1 (*MAD1L1*), Hydroxysteroid 17-Beta Dehydrogenase 11 (*HSD17B11*), Potassium Voltage-Gated Channel Interacting Protein 4 (*KCNIP4*), CaM Kinase Like Vesicle Associated (*CAMKV*), Long Intergenic Non-Protein Coding RNA 1360 (*LINC01360*)
Nievergelt *et al.* (2019)	PTSD.	30,000 cases and 170,000 controls in a multi ethnic cohort	Parkin RBR E3 ubiquitin protein ligase (*PARK2*), Zinc Finger DHHC-Type Containing 14 (*ZDHHC14*), Zinc Finger Protein 813 (*ZNF813*), (Kazrin, Periplakin Interacting Protein (*KAZN*), TMEM51 Antisense RNA 1 (*TMEM51-AS1*), Long Intergenic Non-Protein Coding RNA 2335 (*LINC02335*), microRNA 5007 (*MIR5007*), transcribed ultra-conserved region 338 (*TUC338*), Long Intergenic Non-Protein Coding RNA 2571 (*LINC02571*), major histocompatibility complex, class I, B (*HLA-B*)

PTSD = post-traumatic stress disorder

Combined, these results highlight several important themes. First, it is evident that the genetic etiology of IMDs is complex. The results of the studies conducted thus far have failed to identify consistent specific loci in cohorts. These disparate results may be due to a number of factors. For example, different genes may be involved in distinct processes investigated by specific research questions e.g. the likelihood of having an anxiety disorder versus therapeutic response.. Second, it is possible that inclusion of comorbid disorders, such as in studies that examine both anxiety and depression, may influence the results. Third, this review of GWAS findings included studies that examined a range of anxiety and depressive disorders, and it is possible that focus on specific disorders may yield results that are more likely to be replicated across samples. Finally, studies that examine specific age groups, such as internalizing behaviors in preschool children, may identify age-specific genetic factors.

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Despite these inherent difficulties in cross study comparisons, these results do reinforce several important findings. First, these studies suggest that genetic variants do play a role in the etiology of IMDs, with the possibility of variants implicated in neurotransmission, stress response and inflammatory processes identified. Second, genetic studies need to be conducted in ethnically diverse populations. Bearing this in mind, and given the scarcity of studies conducted in African populations, our genetic investigations in HIV+ children and adolescents in Uganda is both novel and highly relevant.

1.2.4.2 The serotonin transporter gene

Though it has not been found to significantly associate with IMDs in GWASes, the serotonin (5-HT) system has been extensively studied in candidate gene approaches (Table 1.6). Furthermore, the benefit of antidepressant drugs that target the serotonin system in relieving depressive symptoms also supports the value of investigating serotonin-related genes in internalizing mental disorder (IMD) studies (Ernst, Mechawar, & Turecki, 2009). Encoded by the *SLC6A4*, the 5-HT transporter (5-HTT) is a target for SSRIs that competitively block substrate binding and thereby prolong neurotransmitter action at the synapse (Cipriani *et al*., 2018; Kristensen *et al*., 2011). The 5-HTT-linked polymorphic region (*5-HTTLPR*) is located within the promoter region of the *SLC6A4*, 1 kb upstream from the transcription start site on chromosome 17 where it has been reported to influence *SLC6A4* expression (Heils, Mössner & Lesch, 1997; Barca-Garcia *et al*., 2002; Ali *et al*., 2010; Martin *et al*., 2007). The *5-HTTLPR* variant contains two alleles, comprising either the 14 (short, *S*-allele) or the 16 (long, *L*-allele) copies of a 22-23 base pair (bp) imperfect repeat (Heils *et al*., 1996). *In vitro* studies show that the *L*-allele has higher basal transcriptional activity compared to the *S*-allele (Seripa *et al*., 2013; Iurescia *et al*., 2012; Hu *et al*., 2006; Baca-García *et al*., 2002). In close proximity to the *5-HTTLPR* is an *A* to *G* single nucleotide polymorphism (SNP) where the *G*-allele has been reported to alter expression of the *SLC6A4* by creating a functional AP2 transcription-factor binding site (Ehli *et al*., 2012; Hu *et al*., 2006). The *SLC6A4*-rs25531 *L-G* haplotype has been reported to have transcription efficiency equivalent to that of the *S*-allele (Hu *et al*., 2006).

As the therapeutic benefit of SSRIs relies on inhibiting the transporter, and therefore increasing synaptic 5-HT, it is possible that genetic influences on *SLC6A4* expression and thus transporter activity may underlie some of the negative symptoms of IMDs. The *5-HTTLPR S*-allele has been associated with depression (Saul *et al*., 2019; Clarke *et al*., 2010; Wang *et al*., 2016).

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Furthermore, in interaction analyses that account for stress, the *S*-allele has also been associated with elevated risk for depression (Conway, Slavich & Hammen, 2014; Sharpley *et al*., 2014; Karg, Burmeister, Shedden & Sen, 2011; Caspi *et al*., 2003), anxiety disorders (Saul *et al*., 2019; Stein, Schork, & Gelernter, 2008) and PTSD (Li *et al*., 2019; Guo *et al*., 2019; Liu *et al*., 2018; Smoller *et al*., 2016; Gressier *et al*, 2013).

Another widely studied polymorphism within *SLC6A4* is a variable number of tandem repeats polymorphism in intron 2 (*STin2* VNTR), which consists of multiple repeated copies of a 16-17 bp element (Battersby *et al*., 1996; Furlong *et al*., 1998). Two major alleles have been described among a Ugandan sample of PLHIV, containing 10 (*STin2.10*) and 12 (*STin2.12*) copies of this VNTR (Kalungi *et al*., 2017), with the 9-repeat (*STin2.9*) (Ogilvie *et al*., 1996) generally found at a lower frequency. The *STin2* VNTR has been found to interact with the *5-HTTLPR* polymorphism to regulate expression of *SLC6A4* (Ali *et al*., 2010; Haddley *et al*., 2011). Specifically, the *STin2.9* allele directed increased transcription of the *SLC6A4* compared to the *STin2.10* allele, while the *STin2.10* allele directed higher *SLC6A4* transcription than the *STin2.12* allele (Haddley *et al*., 2011; Ali *et al*., 2010). Both *5-HTTLPR* and *STin2* VNTR polymorphisms have been widely investigated in IMDs (Smoller *et al*., 2016). The combination of the *S/S 5-HTTLPR* and *STin2.10* alleles has also been associated with nicotine dependence in Vietnamese men (Koks *et al*., 2018). The *STin2.9* and *STin2.10* alleles have both been found to be associated with anxiety in patients with self-harming behaviors (Evans, Li, & Whipple, 2013) while the *STin2.12* allele has associated with depression, neuroticism and suicide (Kalungi *et al*., 2017;de Lara *et al*., 2007; O'Gara *et al*., 2008). In addiiton, possessing the *STin2.10* allele has been associated with protection against PTSD (Xiao *et al*., 2019). Table 1.6 gives a summary of studies that have been carried out on *SLC6A4 5-HTTLPR* and *STin2* VNTR polymorphisms among IMDs.

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Table 1.6: Studies on *SLC6A4* polymorphisms in internalizing mental disorders

Reference	IMD, Polymorphism	Ethnicity	Study characteristics	Major findings
Conway, Slavich & Hammen, 2014	Depression, 5-*HTTLPR*	C:45.2% L:43.3% B:4.8% A:2.9% NA:1% O:3%	104 undergraduate college students, (73.1% female), mean age 19.64 years	Daily stress levels associated with severity of internalizing symptoms, but only for 5-*HTTLPR S*-allele carriers
Sharpley *et al*., 2014	Depression, 5-*HTTLPR*	NR	Meta-analysis of 81 studies, 55,269 participants, (44.9% female), mean age 36.1 years	*S*-allele associated with depression following any form of stress
Karg, Burmeister, Shedden & Sen, 2011	Depression, 5-*HTTLPR*	NR	Meta-analysis of 54 studies, 40,749 participants	*S*-allele associated with an increased risk of developing depression under stress
Caspi *et al*., 2003	Depression	North American/European white	Dunedin Multidisciplinary Health & Development (DMHD) study of 1,037 children (48% female), a total of 847 participants were selected from the DMHD	*SS* carriers exhibited more depressive symptoms & diagnosable depression in relation to stressful life events than *LL* carriers
Saul *et al*., 2019	Depression 5-*HTTLPR*/rs25531	NR	198 Southern Tasmanian adults with multiple sclerosis, (69.6% female), mean age 48.5 years	Association between stress load & depression stronger among *SS* carriers
Hariri *et al*., 2002	Anxiety	C: 89.3%, AA: 10.7%	28 right-handed healthy volunteers (20 females, 8 males)	*SS* carriers exhibit greater amygdala reactivity in response to

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				fearful stimuli than *LL* carriers
Heinz *et al*., 2005	Anxiety	NR	29 right-handed male volunteers	*S*-allele carriers showed stronger activation of the amygdala when presented with aversive pictures
Munafò *et al*., 2009b	Anxiety	NR	3,872 participants from the Northern Finland Birth Cohort followed by a meta-analysis of 51 studies	*5-HTTLPR* associated with neuroticism
Xiao *et al*., 2019	PTSD, 5-*HTTLPR* & *STin2* VNTR	Chinese	567 Tibetan adolescent earthquake survivors (287 PTSD cases & 280 controls)	*STin2* VNTR but not *5-HTTLPR* associated with PTSD (*STin2.10* negatively correlated with PTSD
Gressier *et al*., 2013	PTSD	NR	A meta-analysis of 12 studies among trauma exposed individuals	Trauma exposed *SS* carriers had higher risk for PTSD
Mushtaq *et al*., 2012	PTSD	NR	Adult outpatient residents of Kashmir, India	*LL* carriers associated with better response to treatment compared *SS* and *SL* carriers among PTSD patients treated with sertraline
Xie *et al*., 2009	PTSD, 5-*HTTLPR*/rs25531	EA:582, AA:670	1,252 individuals who reported experiences of childhood adversity, adult traumatic events, or both, recruited at 4 centers in the US (48%	The *5-HTTLPR* interacted with adult traumatic events & childhood adversity to increase the risk for PTSD. The *S*-allele was a

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			female), mean age 38.5 years	risk factor for PTSD.
Lee *et al*., 2005	PTSD, 5-*HTTLPR*	Korean	100 PTSD patients & 197 unrelated healthy controls, recruited from existing clinical populations in Korea, (57% female), mean age 35.3 years	The frequency of the *SS* genotype was significantly higher in PTSD patients than in normal controls

IMD = internalizing mental disorder, L = Latino, B = mixed race, A = Asian, NA = Native American, O = Others, C = North American/European white, NR = not reported, EA = European American, AA = African American, PTSD = post-traumatic stress disorder, *5-HTTLPR* = serotonin transporter gene-linked polymorphic region

1.2.4.3 The tryptophan hydroxylase 2 gene

The tryptophan hydroxylase enzyme (TPH2) exists as two isoforms, TPH1 and TPH2, which are independently encoded on the *TPH1* and *TPH2* genes (Hasegawa & Nakamura, 2010). TPH2 catalyzes the rate-limiting step in 5-HT biosynthesis (Carkaci-Salli *et al*., 2006; Walther *et al*., 2003) and is found exclusively in the brainstem (Kennedy *et al*., 2012), an area which is the major locus of serotonin-producing neurons (Kennedy *et al*., 2012). Polymorphisms in *TPH2* have been associated with IMDs (Reuter, Kuepper, & Hennig, 2007; Van Den Bogaert *et al*., 2006; Zhang *et al*., 2005). To this end, a meta-analysis of 27 studies that studied 74 SNPs within *TPH2* confirmed the involvement of this gene in depression (Gao *et al*., 2012). rs34517220, an intronic variant located within transcription factor binding sites of *TPH2* (Gassó *et al*., 2017), has also been reported as a functional variant that modulates human *TPH2* expression in antidepressant response to SSRIs (Gassó *et al*., 2017). In addition, rs4570625, located in the 5' regulatory region (Latsko *et al*., 2016), and rs1386494, located approximately 2 kb from the 5' end of *TPH2* (Zill *et al*., 2004), have been associated with reduced transcription rates of *TPH2* (Porcelli *et al*., 2011) and thus may have an effect on 5-HT turnover in the brain.

A functional brain imaging study (eighteen cases and six controls) found evidence for increased 5-HT synthesis in the hippocampus and basal nuclei of participants with social anxiety disorder (Furmark *et al*., 2016). Interestingly, this increased synthesis capacity was linked to the *TPH2* rs457062 *T*-allele. Though these findings were based on only five participants who carried the

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T-allele, and therefore need to be interpreted with caution, they nevertheless suggest a functional impact of this SNP on brain structures relevant to emotional processing (Furmark *et al*., 2016). Moreover, in the longitudinal Estonian Children Personality, Behaviour and Health Study (n = 1234), the rs4570625 *TT* genotype was also associated with reduced prevalence of anxiety disorders at age 25 years (Laas *et al*., 2017).

A study among adult Chinese participants who experienced an earthquake (n = 326) reported the rs11178997 *T*-allele as a significant predictor of severity of PTSD avoidance symptoms in women (Cao *et al*., 2014). In addition, the *T*-allele of rs11178997 was significantly associated with PTSD symptoms among a sample of 200 adults exposed to the 1988 Spitak earthquake from twelve multigenerational families (Goenjian *et al*., 2012). However, findings on the association between *TPH2* variants and PTSD are mixed. A study by Goçi *et al*. (2019) reported no association between *TPH2* rs11178997 and rs1386494 on either depression or PTSD among participants who experienced war-related trauma in Bosnia and Herzegovina, Kosovo and Croatia. It is thus possible that the genotype-dependent effects found by Cao *et al*. (2014) and Goenjian *et al*. (2012) may have been associated with shared qualitative aspects of the trauma experienced following exposure to an earthquake.

As *TPH2* encodes for the rate-limiting enzyme in 5-HT synthesis, there is a strong biological rationale for its investigation as a candidate gene in studies of IMD etiology. This is further supported by evidence for associations between *TPH2* SNPs and depressive disorders, anxiety disorders and PTSD. However, very few of the studies summarized above investigated the interactive effects of stress and genetic variation in *TPH2* on anxiety and depressive disorders. Furthermore, the studies were limited to adult participants. However, the findings of the longitudinal Estonian birth cohort study also suggest *TPH2* variants may influence vulnerability to IMDs during developmental periods (Laas *et al.*, 2017) and may thus be particularly relevant to our examination of IMDs in children and adolescents.

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Table 1.7: Studies on tryptophan hydroxylase 2 gene polymorphisms in internalizing mental disorders

Reference	IMD, Polymorphism	Ethnicity	Study characteristics	Major findings
Reuter, Kuepper, & Hennig, 2007	Anxiety, rs4570625	European white	400 healthy German descent subjects (72% females), mean age 23.7 years	rs4570625 *TT* genotype associated with harm avoidance
Van Den Bogaert *et al*., 2006	rs1178997, Unipolar and bipolar depression	European white	182 patients and 364 controls	rs1178997 protective against both unipolar and bipolar disorder
Zhang *et al*., 2005	Major depression, rs120074175	African-American, North American white	87 cases of unipolar major depression, 219 controls	rs120074175 *A*-allele associated with major depression
Gao *et al*., 2012	Major depressive disorder	North American/European white, Asians, African Americans	Meta-analysis of 27 studies with 74 SNPs	rs4570625 associated with major depressive disorder
Gassó *et al*., 2017	Major depressive disorder & GAD	European white	83 Spanish children and adolescents treated with fluoxetine	rs34517220 associated with reduction in depressive symptoms
Furmark *et al*., 2016	Anxiety, rs4570625	NR	18 anxiety patients (80% female, mean age 35.2 years) and 6 healthy controls (50% female, mean age 22.5 years)	rs4570625 *T*-allele associated with increases 5-HT synthesis rate
Laas *et al*., 2017	rs4570625, Anxiety, Depression	European white	1,176 children from the Estonian children's personality behavior & health study	rs457065 *T*-allele associated with low aggressiveness, low anxiety and low depressiveness
Cao *et al*., 2014	PTSD, rs11178997	Chinese	326 adult Chinese survivors of 2008 Wenchuan earthquake	rs11178997 significantly predicted severity of PTSD's avoidance symptoms

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Goenjian *et al*., 2012	PTSD, depressive symptom, rs11178997	Chinese	200 adults exposed to earthquake	rs11178997 *T*-allele associated with symptoms of PTSD

NR = not reported

1.2.5 Telomere length and internalizing mental disorders

1.2.5.1 Telomeres and telomere length

Targeted investigations of the genetic contributions to IMDs have examined multiple candidates beyond 5-HT. One such candidate, telomere length, is an especially intriguing option for exploration. Telomeres are protein-bound deoxyribonucleic acid (DNA) repeat structures at the ends of chromosomes. They are important in protecting chromosomes from fusing together during mitosis, thus preventing loss of genetic data (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006). They also regulate cellular replicative capacity (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006). During each somatic cell replication, TL progressively shortens due to the inability of DNA polymerase enzyme to fully replicate the 3' end of the DNA strand, a process termed the "end replication problem" (Watson, 1972). This results in a gradual decline in TL over time. Once a critical TL is reached, the cell is triggered to enter a phase of replicative senescence and subsequently cell death (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006).

In germ cells and other stem cells that require replenishing, TL is maintained by a telomerase enzyme component known as telomerase RNA component (TERC) and a reverse transcriptase enzyme known as the telomerase reverse transcriptase (TERT) (Blackburn, Greider, & Szostak, 2006; Wang & Meier., 2004). The ataxia-telangiectasia mutated (ATM) kinase enzyme has also been recently reported to play a role in TL maintenance through phosphorylation and removal of the telomeric repeat-binding factor 1 (TRF1) protein from telomeres (Lee *et al*., 2015). Shorter TL has been related to both dampened telomerase activity (Epel *et al*., 2004) and, paradoxically, elevated telomerase activity (Damjanovic *et al*., 2007). These contradictory results may be due to whether elevated telomerase activity is able to compensate for TL attrition or may be overwhelmed if the causes of the TL attrition are sufficiently potent (Lindqvist, Simon & Wolkowitz, 2019).

Importantly, TL is subject to multiple environmental influences and therefore may offer a biological proxy of the integrated effects of environmental exposures. Of particular relevance

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to this study, TL is influenced by stress exposure and infectious diseases, such as HIV (Epel & Prather, 2018; Shiau *et al*., 2018). As a marker of cellular aging, TL has also been linked to many age-related diseases such as cardiovascular disease (Rehkopf *et al*., 2016) and neurodegenerative disorders (Forero *et al*., 2016), as well as psychopathology (Epel & Pranther, 2018). Studies have associated reduced TL with accelerated cellular aging, age-related disease and reduced years of survival (Sanders & Newman, 2013; Epel *et al*., 2009) while longer telomeres have been associated with increased years of healthy life (Njajou *et al*., 2007).

Telomere length may thus link environmental exposure to risk of IMDs and their associated effects on physical health. Indeed, it has been reported that patients with IMDs have higher mortality rates compared to the general population, and that the mortality is mainly due to the same age-related diseases (such as cancer, heart and cerebrovascular disease) as the general population (Pratt, Druss, Manderscheid & Walker, 2016; Druss *et al*., 2011; Colton & Manderscheid, 2006; Cuijpers & Smit, 2002). The biochemical processes that underlie the comorbidity between IMDs and age-related diseases require investigation and future genetics studies among patients with comorbid IMDs and metabolic diseases may provide insights into the association between IMDs and metabolic disease.

Telomere length has been shown to shorten rapidly from birth to age five years, and then enter a period of relative stability until young adulthood (defined as early 20s), after which more gradual attrition occurs (Wojcicki *et al*., 2016; Frenck, Blackburn & Shannon, 1998). Therefore, identifying TL differences in studies of children and adolescents, an age of expected TL stability and therefore lower variability, could offer a more accurate insight into the causal determinants of the observed changes. The value of studying TL in younger samples is borne out by the numerous investigations reporting associations between adverse childhood experiences and shorter TL (Bürgin *et al*., 2019; Chen *et al*., 2019; Wade *et al*., 2019; Xavier *et al*., 2018).

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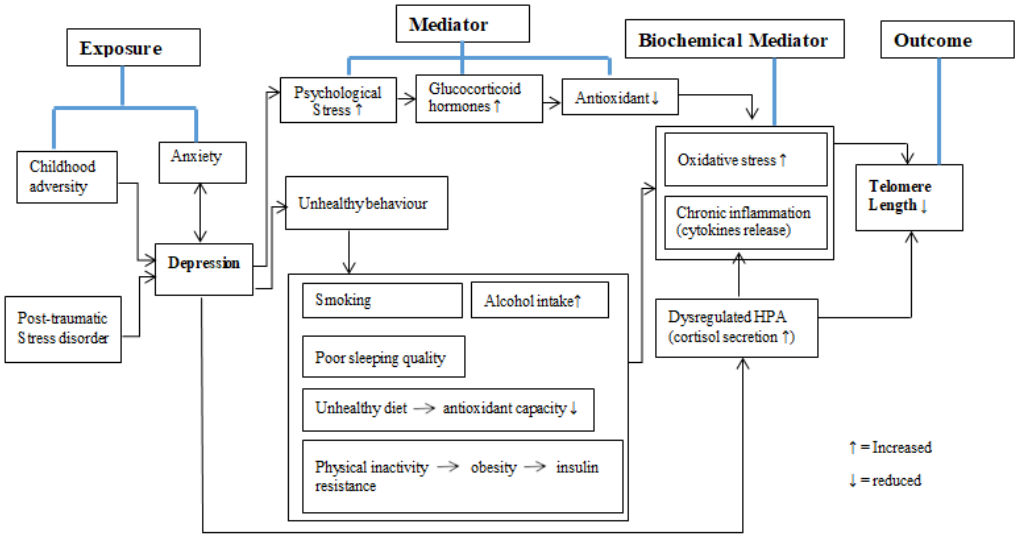

Figure 1.1: A schematic of pathways linking internalizing mental disorders to telomere shortening (Adapted from Yu & Woo, 2016).

In an effort to elucidate mechanisms underlying IMDs, studies have explored the role of TL in the etiology of IMDs. Besides associations with IMDs, TL have also been found to be associated with exposure to chronic stress (Epel *et al*., 2004; Notterman & Mitchell, 2015) and early life stress (Tyrka *et al*., 2016), known risk factors for IMDs (Adelman *et al*., 2014; Evans, Li, & Whipple, 2013; Revenson *et al*., 2016; Robles, Slatcher, Trombello, & McGinn, 2014). These associations point to an underlying biological embedding of TL in the etiology of IMDs that warrant investigation. The following section gives an overview of these associations.

1.2.4.2 Telomere length and etiology of internalizing mental disorders

Several studies in children and adolescents of unknown HIV status have reported associations between experiencing adversity and telomere shortening (Bürgin *et al*., 2019; Mayer *et al*., 2019; Alexeeff *et al*., 2019; Shalev *et al*., 2013; Theall *et al*., 2013; Drury *et al*., 2014; Mitchell *et al*., 2014). Adversity experienced in childhood and adolescence ranges from exposure to traumatic stressors, such as sexual and physical abuse, to social adversities that relate to family structure, parental mental distress, and SES (Shalev *et al*., 2013; Theall *et al*., 2013; Drury *et al*., 2014; Mitchell *et al*., 2014). Living with HIV has also been reported as a potential source of trauma among both adults (Nightingale *et al*., 2011), and children and adolescents (Ahlers, 2016).

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Many studies have associated IMDs with shorter TL (Avetyan, Zakharyan, Petrek & Arakelyan, 2019; Malouff & Schutte, 2017; Verhoeven *et al*., 2014, 2015, 2017; Lin, Huang & Hung, 2016; Garcia-Rizo *et al*., 2013; Shalev *et al*., 2014; Zhang *et al*., 2014; Douillard-Guiloux *et al*., 2013; Kinser *et al*., 2013; Kananen *et al*., 2010; O'Donovan *et al*., 2011). Among adult samples, shorter TL has been associated with depression (Lin, Huang & Hung, 2016; Schutte & Malouff, 2015), anxiety disorders (Verhoeven *et al*., 2015, 2017; Malouff & Schutte, 2017) and PTSD (Avetyan, Zakharyan, Petrek & Arakelyan, 2019; O'Donovan *et al*., 2011; Zhang *et al*., 2014). Although the nature of the association between IMDs and TL is not well known, several biological mechanisms have been proposed. IMDs have been reported as chronic stressors (McEwen, 2003), which can lead to long-term alterations to the hypothalamic-pituitary-adrenal (HPA) axis, and thus impact on the stress response. In the first study to examine the associations between TL, stress reactivity and risk for depression, Gotlib *et al*. (2015) found that shorter TL was associated with increased stress-induced cortisol reactivity in children born to mothers with a history of recurrent depressive episodes.

Internalizing mental disorders could also result in TL attrition through inflammatory pathways. Depression has, for example, been reported to prime massive cytokine responses to stressors (Kiecolt-Glaser, Derry & Fagundes, 2015). Increased systemic inflammation has also been associated with decreased TL among a prospective cohort of workers exposed to high levels of fine particulate matter (Wong *et al*., 2014). Finally, both HPA axis dysregulation and inflammation have been linked to increased oxidative stress, which has emerged as a common finding across a range of IMDs and which is in turn associated with TL attrition (Shafiee *et al*., 2018; Czarny, Wigner, Galecki & Sliwinski, 2018; Miller, Lin, Wolf & Miller, 2018; Spiers, Chen, Sernia, & Lavidis, 2015; Epel & Lithgow, 2014). Increased oxidative stress (Yu & Woo, 2016) has been proposed as the biological processes through which IMDs lead to accelerated TL shortening (Figure 1.1).

IMDs may thus be mediating accelerated TL attrition, or accelerated TL attrition may causally be involved in IMDs (Yu & Woo, 2016). Table 1.8 summarizes various studies that have investigated the association between different IMDs and TL.

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1.2.4.2 Genetic polymorphisms and telomere length

Since TL is partially genetically determined, with heritability estimates ranging from 44% to 80%, and highly variable between individuals (Njajou *et al*., 2007; Vasa-Nicotera *et al*., 2005), it is likely that genetic variation contributes to telomere maintenance (Codd *et al*., 2013). This genetic variation may present as a stable marker confering risk for IMDs or accelerated TL attrition. In an effort to identify loci that affect mean TL, a genome-wide meta-analysis that involved 37,684 individuals of European descent and replication in 10,739 European individuals was carried out, in which numerous loci were identified as being associated with mean TL: rs2736100 of *TERT*, rs755017 of regulator of telomere elongation helicase 1 gene (*RTEL1*), rs11125529 of acyclophosphatase-2 gene (*ACYP2*), rs10936599 *TERC*, rs7675998 of nuclear assembly factor 1 gene (*NAF1*), rs9420907 of oligonucleotide/oligosaccharide binding fold 1 gene (*OBFC1*) and rs8105767 of zinc finger 208 gene (*ZNF208*).

Within *TERT*, rs10069690 has been reported to regulate expression of the gene, rs2736100 has been associated with shorter TL among individuals of European descent from fifteen cohorts (Codd *et al*., 2013) and rs7726159 and rs2853669 have been associated with longer TL among participants from Seoul and Copenhagen respectively (Ko *et al*., 2016; Rode, Nordestgaard & Bojesen, 2016). In *TERC*, rs16847897 and rs12696304 have been associated with shorter TL among participants of European descent (Codd *et al*., 2010; Melin, Nordfjäll, Andersson & Roos, 2012), while rs10936599 has been associated with longer TL (Jones *et al*., 2012). *TERT* rs2736100 has also been associated with PTSD and the *T*-allele has been reported to confer risk for PTSD in an Armenian population (Avetyan, Zakharyan, Petrek & Arakelyan, 2019).

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Table 1.8 Studies investigating the association of telomere length and internalizing mental disorders

Reference	IMD	Population characteristics	N	Study design	Major findings
Verhoeven, Penninx & Milaneschi, 2019	Depression	Adults from the Netherlands Study of Depression and Anxiety (NESDA)	2,032	GWAS	PRS for depression was associated with lifetime depression but not TL; PRS for TL associated with TL & 6-year TL attrition rate
Avetyan, Zakharyan, Petrek & Arakelyan, 2019	PTSD	Adult Armenian combat veterans	90	Case-control	PTSD patients had shorter TL compared to controls; rs2736100 *T*-allele a risk factor for PTSD
Chang *et al*., 2018	Depression & anxiety	Adult female European ancestry participants from the US's Nurses' Health Study in the	17,693	Polygenic risk score	Genetic predisposition to shorter TL not a risk factor for depression and anxiety
Michalek *et al.*, 2017	Depression	Adult UK sample	2,768	Mendelian randomization	*T*-allele of rs10936599, a SNP near the telomerase RNA component, predicted increased risk for childhood-onset depression
Lin, Huang & Hung, 2016	Depression	3,414 cases and 3,793 controls	7,207	Meta-analysis	Depression associated with shorter TL

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Shalev *et al*., 2014	Depression, anxiety & PTSD	Adults samples from Dunedin Multidisciplinary Health and Development Study	1,037	Prospective	Persistence of IMDs predicted shorter TL
Wolkowitz *et al*., 2011a	Depression	18 subjects with depression and 18 controls matched by sex, age and ethnicity	36	Case-control	TL inversely correlated with lifetime depression exposure
Verhoeven *et al*., 2017	Depression & anxiety	Adult participants from the Netherlands Study of Depression and Anxiety	2,936	Prospective cohort	Lifetime depression or anxiety associated with shorter TL
Malouff & Schutte, 2017	Anxiety	17 different samples	19,424	Meta-analysis	Higher anxiety associated with shorter TL
Verhoeven *et al*., 2015	Anxiety disorder	Adult participants from the Netherlands Study of Depression and Anxiety	2,324	Case-control	Anxiety disorders associated with accelerated cellular ageing, which in part may be reversible after remission
Schutte & Maouff, 2015	Depression	Systematic review on the relationship between depression and TL	21,040	Meta-analysis	Depression associated with shorter TL
Yu & Woo, 2016	Depression	Systematic review study	NR	Systematic review	Telomeres link depression to accelerated cellular aging

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Simon *et al*., 2006	Anxiety, Depression	Adults with depression or anxiety, with age-matched controls, recruited at MGH.	88	Case-control	TL was significantly shorter in those with mood disorders, representing as much as 10 years of accelerated aging
Verhoeven *et al*., 2014	Depression	Data on adult samples from the Netherlands Study of Depression and Anxiety	2,407	Case-control	Depressed patients show accelerated cellular aging according to a 'dose–response' gradient
Shin, Shin & Lee, 2019	Depression	Young adult data from the National Health and Nutrition Examination Survey (NHANES), United States	1,141	Case-control	A significant and decreasing linear trend in TL was found as CRP levels increased in men, regardless of the depression status, and women with major depression or depressed affect
Roberts *et al*., 2017	PTSD	Probable PTSD cases & matched controls (all trauma exposed), from the Nurses' Health Study 11 in the US.	2,112	Case-control	PTSD was associated with shorter TL

CVD = cardiovascular disease; CRP = C-reactive protein; GWAS = genome-wide association study; MGH = Massachusetts General Hospital; PTSD = post-traumatic stress disorder; SNP = single nucleotide polymorphism; TL = telomere length

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1.2.5 Telomere length and human immunodeficiency virus infection

In addition to stress exposures, TL is also heavily influenced by a range of biological factors, including infectious disease such as HIV. Evidence from a range of studies (summarized in Table 1.9) provides evidence for shorter TL in people with HIV compared to uninfected peers. Although nucleoside reverse transcriptase inhibitors have been reported to inhibit human telomerase *in vitro* (Hukezalie *et al*., 2012; Leeansyah *et al*., 2013), no associations have been reported between TL and exposure to nucleoside reverse transcriptase inhibitors *in vivo* (Solomon *et al*., 2014; Montejano *et al*., 2017). Studies have in fact associated exposure to combination antiretroviral therapy with longer TL in individuals living with HIV among participants who were predominantly of European descent, recruited from 15 European countries (Montejano *et al*., 2018; Stella-Ascariz *et al*., 2018). The gain in TL due to ART in these two studies has been suggested to be due to immune reconstitution, presumably resulting in a larger subset of less mature T cells harboring longer telomeres and, hence, increased TL (Côté & Hsieh, 2018).

Table 1.9: Studies of the association between HIV-infection and telomere length

Reference	Study characteristics	Key findings
Alejos *et al*., 2019	201 randomly selected samples belonging to ART naïve patients from 15 European countries	HIV-infection and age associated with shorter TL
Auld *et al*., 2016	Ugandan patients with suspected tuberculosis (118 HIV infected patients against 66 HIV-uninfected patients)	HIV-infection associated with shorter TL
Pathai *et al*., 2013	South African Xhosa patients (200 HIV infected against 200 HIV-uninfected patients, median ages 39 and 40 respectively)	HIV-infection associated with shorter TL
Zanet *et al*., 2014	Canadian adults (220 HIV-infected against 199 HIV-uninfected participants, aged 20 - 76 years)	HIV-infection associated with shorter TL
Srinivasa *et al*., 2014	United States adults (102 HIV-infected against 41 HIV-uninfected men, aged 18 – 55 years)	HIV-infection associated with shorter TL
Shiau *et al*., 2018	South African children in a cohort study (120 HIV-infected, 33 HIV-exposed uninfected and 25 HIV-unexposed uninfected, mean age 6.4 years)	TL shorter among HIV-infected children compared with HIV-unexposed uninfected children.
Gianesin *et al*., 2016	71 HIV-infected (HIV+), 65 HIV-exposed-uninfected (HEU), and 56 HIV-unexposed-uninfected (HUU) children, aged 0-5 years	Shorter TL in HIV+ than in HEU and HUU children

HEU = HIV-exposed uninfected; HIV = human immunodeficiency virus; HUU = HIV-exposed uninfected; TL = telomere length

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Though shortening of TL has been reported to occur early after infection (Gonzalez-Serna *et al*., 2017), the mechanisms through which TL changes following acute HIV infection or ART initiation are yet to be elucidated. However, long-term TL attrition could occur partially as a result of the chronic stress that is associated with HIV infection. Chronic stress has been linked to shorter telomeres (Damjanovic *et al*., 2007; Epel *et al*., 2004; Notterman & Mitchell, 2015; Parks *et al*., 2009; Shalev, 2012; Shalev *et al*., 2013). TL has therefore been proposed as a marker for chronic stress (Needham *et al*., 2015; Zhang *et al*., 2014; Houben, Moonen, van Schooten, & Hageman, 2008). As HIV/AIDS may be viewed as a chronic psychological stressor due to the illness and stigma that are associated with the disease (Varni, Miller, McCuin & Solomon, 2012), this offers a further line of evidence to support TL attrition in HIV infection (Alejos *et al*., 2019; Auld *et al*., 2016; Gianesin *et al*., 2016; Oeseburg, de Boer, van Gilst & van der Harst, 2010; Pathai *et al*., 2013; Shiau *et al*., 2018; Srinivasa *et al*., 2014; Zanet *et al*., 2014).

1.2.6. Justification for the present study

The diathesis-stress hypothesis of neuropsychiatric disorders postulates that a lower stress threshold is required for psychiatric disease to occur in individuals who harbor certain vulnerability factors, which may be genetic and/or acquired (Caspi *et al*., 2003; Silberg *et al*., 2001; Monroe & Simons, 1991). Given the highlighted role of stress in IMDs, the conceptual framework of the present study was based on the diathesis-stress hypothesis. We postulated that acute stress leads to IMDs through vulnerability among Ugandan HIV+ children and adolescents.

Vulnerability was considered in terms of both genetic and acquired factors. IMDs that occur in early life show higher level of familial aggregation than those with a later onset (Rapee, 2018; Wickramaratne & Weissman, 1998; Weissman *et al*., 1984), hence the importance of molecular genetic studies of childhood-onset IMDs.

Since they encode components of the serotonergic neurotransmission pathway, *SLC6A4* and *TPH2* represent ideal candidates to investigate in IMDs. IMDs have been associated with polymorphisms within the *SLC6A4* (Saul *et al*., 2019; Sharpley *et al*., 2014; Conway, Slavich & Hammen, 2014; Arango *et al*., 2001; Malison *et al*., 1998) and *TPH2* (Gassó *et al*., 2017; Gao *et al*., 2012; Reuter, Kuepper, & Hennig, 2007; Van Den Bogaert *et al*., 2006). Polymorphisms within these genes may present as either vulnerability or resilience factors for

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IMDs. We further investigated *SLC6A4* and *TPH2* as potential sources of genetic vulnerability to IMDs by assessing the moderating role of selected polymorphisms within these genes on the association between acute stress and IMDs.

Numerous studies discussed above have reported on the robust association between TL and IMDs (Verhoeven *et al*., 2015; Verhoeven *et al*., 2014; O'Donovan *et al*., 2011; Shalev *et al*., 2014; Gotlib *et al*., 2015; Malan *et al*., 2011; Zhang *et al*., 2014). Some studies have suggested that accelerated telomere shortening is a risk factor for IMDs (Gotlib *et al*., 2015; Malan *et al*., 2011; Shalev *et al*., 2014), while others have found that the development of IMDs and shortening of telomeres are simultaneous effects of increased stress exposure (Zhang *et al*., 2014). We hypothesized that shorter TL would be associated with IMDs in our population, and that genetic variants would moderate the association between TL and IMDs. To test this hypothesis, we investigated the association between TL at IMDs both at baseline and after 12 months. As genetic variations within the *TERC* and *TERT* have also been reported both confer risk for IMDs (Avetyan, Zakharyan, Petrek & Arakelyan, 2019) and influence mean TL (Codd *et al*., 2013), the present study sought to understand whether genetic variations in these genes would moderate the association between TL and IMDs.

With respect to acquired vulnerability, we used the results of previous studies to guide our selection of factors to investigate. In line with previous studies that have reported chronic stress as a risk factor for IMDs (Adelman *et al*., 2014; Evans, Li, & Whipple, 2013; Revenson *et al*., 2016; Robles, Slatcher, Trombello, & McGinn, 2014), the present study considered chronic stress as an acquired vulnerability factor for IMDs in the conceptual framework.

1.3 Problem statement

The etiology of IMDs is poorly understood. There are currently no reliable biomarkers for IMDs, and diagnosis is based on clinical symptoms, increasing the possibility of misdiagnosis. This is especially so given that patients with the same underlying pathophysiology may present differently, as well as the possibility of misinterpretation of symptoms and signs due to cultural differences. Understanding the genetic and environmental risk factors for IMDs is central in providing insights into pathways that could be involved in the etiology of IMDs. Understanding the etiology of IMDs will inform the development of new more therapeutically meaningful drugs and drug targets and may help identify persons at increased risk for IMDs for early intervention strategies.

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1.4 Hypotheses

Our overarching hypothesis is that acute stress leads to IMDs through vulnerability (chronic stress or TL and genetic variants in *SLC6A4* and *TPH2*). We further hypothesized that shorter TL is associated with the occurrence of IMDs among HIV+ children and adolescents and that this association is moderated by vulnerability (chronic stress or genetic variants in *TERT and TERC*).

1.5 Study objectives

1.5.1 General aim

The primary aim of the present study was to determine the association between acute stress and internalizing mental disorders and investigate factors that moderate this relationship, among HIV+ children (aged 5-11 years) and adolescents (aged 12-17 years) in Uganda.

We **hypothesized** that there would be a significant association between acute stress exposure and IMDs and that this would be moderated a vulnerability factor (genetic and/or acquired). Although we hypothesized that shorter TL would be associated with the occurrence of IMDs, the nature of association between TL and IMDs, we sought to first investigate that association to determine whether to model TL as a vulnerability factor for IMDs (Figure 1.2, F).

1.5.2 Objectives

1) To investigate the association between relative TL and IMDs among HIV+ children and adolescents in Uganda and whether this association is moderated by vulnerability.

1.1 To investigate the longitudinal association between IMDs and TL (Figure 1.2, F).

1.2 To determine whether the association in 1.1 (if any) is moderated by vulnerability (genetic and/or acquired) (Figure 1.2, G).

2) To investigate the association between acute stress and IMDs among HIV+ children and adolescents in Uganda and whether this association is moderated by vulnerability (Figure 1.2, B).

2.1 To investigate the association between acute stress and IMDs (Figure 1.2, A).

2.2 To investigate whether the association in 2.1 (if any) is moderated by acquired vulnerability (chronic stress, TL) (Figure 1.2, E).

2.3 To investigate whether the association in 2.1 (if any) is moderated by genetic vulnerability (*SLC6A4* and *TPH2* polymorphisms) (Figure 1.2, C).

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1.6 Conceptual framework

The conceptual framework for the present study, as shown in Figure 2 was adapted from the diathesis-stress hypothesis for depression by Monroe & Simons (1991), which states that depression will occur when stress acts on an underlying vulnerability (genetic or acquired).

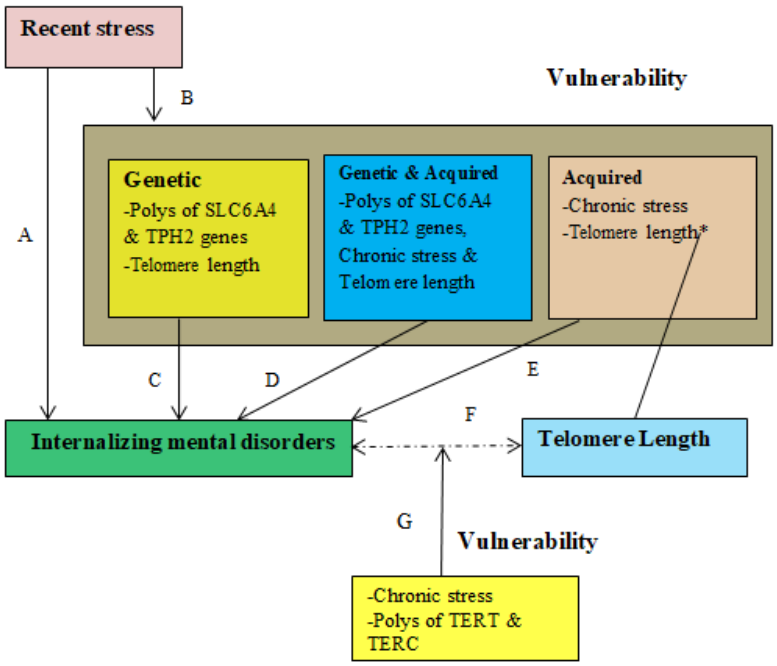

Figure 1.2: The conceptual framework of the study, based on the diathesis-stress hypothesis.

The conceptual model for the present study was based on the diathesis-stress model for depression by Monroe & Simons (1991), that states that depression will only occur when stress acts on an underlying vulnerability (genetic or acquired). In line with the diathesis-stress model, we postulated that acute stress leads to IMDs through vulnerability (B).

*Vulnerability can be acquired (E; **Objective 2.2**) or genetic (C; **Objective 2.3**), or an individual may have both genetic and acquired vulnerabilities (D).*

*The aim of the present study was to investigate the association between acute stress and IMDs (A; **Objective 2.1**), and to determine whether this association was moderated by vulnerability factors (B), namely, genetic factors, TL and/or chronic stress exposure. In addition, we investigated the association between TL and IMD diagnosis (dashed line (F); **Objective 1.1**),*

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an association we hypothesized would be moderated by vulnerability factors (genetic and chronic stress exposure) (G; ***Objective 1.2****).*

1.7 Layout of the thesis

Chapter 2 contains a manuscript that answers **Objective 1.1** of the study. The manuscript is entitled: "*Internalizing mental disorders and accelerated cellular aging among perinatally HIV-infected youth in Uganda*". Work contained in this manuscript was published in *Frontiers in Genetics* (see Addendum B for the web version of the article). The PhD candidate played the following roles in the publication of the article: conceived the idea of the study, participated in data collection, carried out TL assays, participated in data analysis, prepared both the first draft and the final revised copy.

Chapter 3 contains a manuscript that answers **Objective 1.2** of the study. The manuscript is entitled: "*The rs2736100 within the telomerase reverse transcriptase gene and rs16847897 within the telomerase RNA component gene moderate the association between internalizing mental disorders and accelerated telomere length attrition among HIV+ children and adolescents in Uganda*". This manuscript has been submitted to *BMC Medical Genetics* and is currently editorial assessment. The PhD candidate played the following roles in the preparation of the manuscript: conceived the idea for the study, participated in data collection, carried out TL assays, participated in data analysis, and prepared both the first draft and the final revised copy.

Chapter 4 contains a manuscript that answers **Objectives 2.1 – 2.3** of the study.
The manuscript is entitled: "*The 5-HTTLPR/rs25531 S-A-S-A haplotype and chronic stress moderate the association between acute stress and internalizing mental disorders among HIV+ children and adolescents in Uganda*". This manuscript is yet to be submitted to a peer-reviewed journal for publication. The PhD candidate played the following roles in the study; conceived the idea of the study, participated in data collection, carried out laboratory assays for genotyping the serotonin transporter gene polymorphisms, constructed a composite index for both acute and chronic stress, participated in data analysis, prepared both the first draft and the final revised copy.

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CHAPTER TWO

This chapter reports the association between IMDs and TL among the study participants. It is a modified version of the publication in *Frontiers in Genetics* (Impact factor 3.517) (See Addendum B for the web version of the article).

Internalizing mental disorders and accelerated cellular aging among perinatally HIV+-infected youth in Uganda

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2.1 Abstract

Introduction: Internalizing mental disorders (IMDs) in HIV+ children and adolescents are associated with impaired quality of life and non-adherence to anti-retroviral treatment. Telomere length is a biomarker of cellular aging, and shorter telomere length has been associated with IMDs. However, the nature of this association has yet to be elucidated.

Objective: We determined the longitudinal association between IMDs and relative telomere length (rTL) and the influence of chronic stress among Ugandan perinatally HIV-infected youth (PHIY).

Methods: IMDs (depressive disorders, anxiety disorders, and post-traumatic stress disorder) were assessed using the locally adapted Child and Adolescent Symptom Inventory-5. In 368

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PHIY with any IMD and 368 age- and sex-matched PHIY controls without any psychiatric disorder, rTL was assessed using quantitative polymerase chain reaction. Hierarchical cluster analysis was used to generate the three chronic stress classes (mild, moderate, and severe). *T-tests* were used to assess the difference between baseline and 12 month rTL and the mean difference in rTL between cases and controls both at baseline and at 12 months. Linear regression analysis was used to model the effects of chronic stress on the association between IMDs and rTL, controlling for age and sex.

Results: We observed longer rTL among cases of IMDs compared with controls ($p < 0.001$). We also observed a statistically significant reduction in rTL between baseline and 12 months in the combined sample of cases and controls ($p < 0.001$). The same statistical difference was observed when cases and controls were individually analyzed ($p < 0.001$). We found no significant difference in rTL between cases and controls at 12 months ($p = 0.117$). We found no significant influence of chronic stress on the association between IMDs and rTL at both baseline and 12 months.

Conclusion: rTL is longer among cases of IMDs compared with age- and sex-matched controls. We observed a significant attrition in rTL over 12 months, which seems to be driven by the presence of any IMDs. There is a need for future longitudinal and experimental studies to understand the mechanisms driving our findings.

Keywords: Internalizing mental disorders, relative telomere length, HIV+, perinatally HIV-infected youth, Uganda

2.2 Background

Human immunodeficiency virus/acquired immunodeficiency disease syndrome (HIV/AIDS) is a significant global health burden, with approximately 36.9 million people infected globally (UNAIDS, 2018). Both eastern and southern Africa remain the most affected regions, accounting for 45% of the world's HIV infections (UNAIDS, 2018). Of the over 2 million HIV-positive (HIV+) children globally, 90% reside in sub-Saharan Africa (UNAIDS, 2010). In Uganda, the country with the fifth-highest HIV prevalence in the region, an HIV prevalence of 0.5% has been reported among children aged 0–14, which corresponds to approximately 95,000 children living with HIV in the country (UPHIA, 2016–2017). The introduction of antiretroviral therapy (ART) has led to improved survival of HIV-infected youth (7–17 years); however, the mental health of these youth has received less attention (Mupambireyi *et al*., 2014). Perinatally HIV-infected youth (PHIY) are faced with a burden of psychiatric morbidity (Kamau *et al*., 2012), in addition to delayed motor and cognitive development (Le Doaré *et al*.,

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2012; Van Rie *et al*., 2007). Studies undertaken in both the developed (Europe and the United States) and developing world (sub-Saharan Africa) have documented depression rates of between 12.7% and 40% (Musisi and Kinyanda, 2009; Gadow *et al*., 2012; Kamau *et al*., 2012; Mellins *et al*., 2012; Nachman *et al*., 2012; Lwidiko *et al*., 2018; Kim *et al*., 2014) among PHIY. For anxiety disorders, rates of 9% to 32.2% have been reported among PHIY (Kamau *et al*., 2012; Mellins *et al*., 2012; Nachman *et al*., 2012; Kinyanda *et al*., 2019).

IMDs are associated with psychological distress (Musisi and Kinyanda, 2009), impaired quality of life, and non-adherence to ART (Walkup *et al*., 2009; Malee *et al*., 2011). In addition, patients with IMDs have higher mortality rates than have the general population (Cuijpers and Smit, 2002; Colton and Manderscheid, 2006; Ahmadi *et al*., 2011; Druss *et al*., 2011).

IMDs are characterized by quiet, internal distress (Tandon, Si & Luby, 2011), in contrast to externalizing disorders, where overtly socially negative or disruptive behavior is displayed (Tandon, Si & Luby, 2011). IMDs with high levels of negative affectivity include depressive disorders (e.g., dysthymic disorder), anxiety disorders (e.g., generalized anxiety disorder and social anxiety disorder), and obsessive-compulsive disorder (Regier *et al*., 2013; Turygin *et al*., 2013). Despite intensive research, the diagnosis of IMDs is still largely based on clinical symptoms, with an absence of biological markers to facilitate diagnosis. This is largely because the pathophysiological mechanisms underlying IMDs, such as depression and anxiety, are still largely unknown. Several studies have investigated the association between telomere length (TL) and IMDs, and shorter TL has been reported in adults with depression (Simon *et al*., 2006; Verhoeven *et al*., 2014; Cai *et al*., 2015b) and anxiety disorders (Kananen *et al*., 2010; Verhoeven *et al*., 2015).

Telomeres are protein-bound deoxyribonucleic acid (DNA) repeat structures at the ends of chromosomes (Lindqvist *et al*., 2015), and are important in preventing chromosomes from fusing together during mitosis, thus preventing loss of genetic data (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006). They also regulate cellular replicative capacity (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006).

During somatic cell replication, telomeres progressively shorten due to the inability of DNA polymerase enzyme to fully replicate the 3′ end of the DNA strand (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006), a process termed as the “end replication problem” (Watson, 1972). This results in a gradual decline in telomere length (TL) over time. Once a

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critically short TL is reached, the cell is triggered to enter replicative senescence and subsequently cell death (Allsopp *et al*., 1992; Blackburn, Greider, & Szostak, 2006). TL provides a metric of cellular age and accounts for roughly 15% of the variance of age (Epel and Prather, 2018). TL has been reported to shorten in a predictable way with chronological age by roughly 20–40 base pairs per year (Cesare and Reddel, 2010). TL is partially genetically determined, with heritability estimates ranging from 36% to 84% (Aviv, 2012) and is highly variable between individuals (Vasa-Nicotera *et al*., 2005; Njajou *et al*., 2007). The present study assessed TL as relative TL (rTL), with rTL being proportional to an individual's TL (Cawthon, 2009).

Several studies in youth have reported associations between adversity and telomere shortening (Shalev *et al*., 2013; Theall *et al*., 2013; Drury *et al*., 2014; Mitchell *et al*., 2014). Adversity experienced in youth ranges from exposure to traumatic stressors, such as sexual and physical abuse, to social adversities that relate to family structure, parental mental distress, and socio-economic status (SES). Causal associations between stressful life events and early adversities, such as childhood sexual abuse and major depression, are well documented (Kendler *et al*., 1999; Fergusson and Mullen, 1999; Kendler *et al*., 2000), with evidence suggesting molecular signatures of stress overlap with major depression (Cai *et al*., 2015b). Biological processes, such as inflammation and oxidative stress, which have been observed in several psychiatric disorders are also associated with telomere shortening (Wolkowitz *et al*., 2011a; Wolkowitz *et al*., 2011b), suggesting that telomere shortening may be related to certain psychiatric endophenotypes.

Depression has been considered a syndrome of accelerated aging (Heuser, 2002). The first study to examine leucocyte TL (LTL) in a group of subjects with either major depression or bipolar disorder and aged-matched controls found shorter LTL among cases compared with healthy controls (Simon *et al*., 2006). A large longitudinal clinical cohort study found shorter LTL among groups who were currently depressed or had remitted depression compared with healthy controls (Verhoeven *et al*., 2014). However, there was no statistically significant difference in LTL between the currently depressed and remitted depression groups, suggesting that depression may leave an "indelible marker" on LTL. However, in the currently depressed group, a dose–response relationship was observed, with LTL inversely associated with both severity and duration of depression. This dose–response relationship was further supported by a longitudinal study by Shalev *et al*. (2014), where persistence of IMDs from 11 to 38 years

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predicted reduced LTL at 38 years of age in a dose-dependent manner among male participants. It is, however, not possible to rule out that LTL was already reduced at the first episode of depression, indicating that shorter LTL could be a risk factor for depression. Indeed, Gotlib *et al*. (2015) described shorter LTL as a risk marker for depression, where shorter LTL was observed among girls (aged 10–14 years) at increased risk for depression. High risk for depression was assessed as having a mother with a history of recurrent episodes of depression, while low risk was assessed as having a mother with no current or past Axis I disorder during a girl's lifetime. However, results across studies have been inconsistent. While several other studies have reported shorter LTL among currently depressed individuals compared with controls (Lung *et al*., 2007; Hoen *et al*., 2011; Wikgren *et al*., 2012; Garcia-Rizo *et al*., 2013), some studies have failed to find an association (Wolkowitz *et al*., 2011a; Teyssier *et al*., 2012; Needham *et al*., 2015; Schaakxs *et al*., 2015).

Accelerated aging has also been described in anxiety disorders. Using the same study population as described in Verhoeven *et al*. (2014), the authors reported shorter LTL among subjects with a diagnosis of current anxiety disorder than among controls (Verhoeven *et al*., 2015). There was, however, no statistically significant difference in LTL between the remitted anxiety disorder group and controls, suggesting that LTL shortening in anxiety disorders may be more reversible than that associated with depression. Needham *et al*. (2015) reported an association between shorter LTL and a diagnosis of generalized anxiety disorder and panic disorder among women. Kananen *et al*. (2010) reported shorter LTL among older anxiety disorder subjects (48– 87 years of age) compared with controls, and a study by Okereke *et al*. (2012) reported a dose–response relationship where severe phobia was associated with shorter LTL.

PTSD has also been considered in the context of accelerated aging (Moreno-Villanueva *et al*., 2013; Miller and Sadeh, 2014). Shorter LTL has been implicated in PTSD, though the effects were primarily explained by early life stress (O'Donovan *et al*., 2011). Shorter LTL was reported among combat-deployed soldiers with PTSD, compared with those without PTSD (Zhang *et al*., 2014). There is a need to understand whether telomere shortening is a direct effect of PTSD, whether the development of PTSD and shortening of telomeres are simultaneous effects of increased stress reactivity (Zhang *et al*., 2014), or whether telomere shortening is a risk factor for PTSD (Malan *et al*., 2011).

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HIV infection has also been found to be associated with shortened telomeres (Oeseburg *et al*., 2010; Auld *et al*., 2016). HIV/AIDS may be viewed as a chronic psychological stressor due to the illness and stigma that are associated with the disease (Varni *et al*., 2012). Since TL has been found to be a marker for chronic stress (Needham *et al*., 2015), shorter telomeres are expected in HIV/AIDS subjects as compared with the disease-free population.

We hypothesized that in PHIY in Uganda, attrition in rTL over a 12-month period would be greater in cases of IMDs compared with age- and sex-matched controls without any psychiatric disorder. We further hypothesized that cases would have shorter rTL than controls. We thus aimed to determine the longitudinal association between IMDs and rTL and the influence of chronic stress in this relationship.

2.3 Methods

2.3.1 Study design

This case–control study was nested within a Medical Research Council/Department for International Development (MRC/ DfID)-funded project that investigated mental health among children and adolescents living with HIV/AIDS in Kampala and Masaka districts of Uganda (CHAKA study), which enrolled 1,339 Ugandan PHIY (7–17 years) of black African ancestry (Kinyanda *et al*., 2019). All participants with any of the IMDs (368 cases) and an equal number of age- and sex-matched controls (n – 368) were selected from CHAKA (N = 736) and included in the present study. Both the baseline and 12-month archived blood sample for each of the included participants was retrieved from which genomic DNA was extracted.

2.3.2 Study population

Study participants were recruited from two HIV clinics in urban Kampala [Joint Clinical Research Centre (JCRC) and Nsambya Home Care] and three HIV clinics in rural Masaka [The AIDS Support Organization (TASO), Kitovu Mobile Clinic, and Uganda Cares]. All study participants were on ART.

2.3.3 Procedures

Consenting PHIY, as well as their caregivers, were interviewed using a structured questionnaire. The questionnaire included, among others, socio-demographic characteristics (sex, study site, age, caregiver level of education, and SES), and modules on depression, post-traumatic stress disorder, and anxiety modules from the DSM-5 referenced Children and

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Adolescent Symptom Inventory-5 (CASI-5) (Gadow & Sprafkin, 2013). The CASI-5 was locally adapted for use in Uganda (Mpango *et al*., 2017). Trained psychiatric nurses and psychiatric clinical officers administered the CASI-5 at two time points (baseline and 12 months). The CASI-5 lists the symptoms of a wide range of psychiatric disorders including major depressive disorder, generalized anxiety disorder, PTSD, and attention-deficit/hyperactivity disorder, among others. Individual CASI-5 items are rated on a 4-point frequency of occurrence scale ranging from never (0) to very often (3). There are several CASI-5 scoring algorithms; however, in the present study we used symptom count cutoff scores that reflect the prerequisite number of symptoms for a clinical diagnosis. Disease severity was assessed by summing up the scores for the symptoms, where a higher score represented severe disease status. At each study visit, 4 ml of blood was withdrawn from each study participant through venipuncture into an EDTA vacutainer and was stored at −80 °C pending DNA extraction.

2.3.4 Inclusion and exclusion criteria

Inclusion criteria: i) HIV-infected outpatients, registered with any of the HIV clinics at any of the study sites; ii) aged between 7 and 17 years at the time of enrolment; iii) conversant in English or Luganda, the language into which the research assessment tools were translated; and iv) able to provide written informed consent(caregivers)/assent(adolescents). Cases were subjects who had any depressive disorder [depression or dysthymia (persistent depressive disorder)] or anxiety disorder. Controls were age-and sex-matched without any psychiatric disorder. Persistent IMDs were baseline cases that remained cases at 12 months, while remitted ones were baseline cases that lost disease status at 12 months *Exclusion criteria*: i) Seriously ill including being unable to understand study procedures and ii) any other psychiatric disorder other than the ones listed above.

2.3.5 Ethical considerations

Both CHAKA and the present study were conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The CHAKA study obtained ethical and scientific clearance from the Uganda Virus Research Institute (UVRI) Science and Ethical Committee (#GC/127/15/06/459) and the Uganda National Council of Science and Technology (#HS 1601). The present study obtained approval from the Higher Degrees Research & Ethics Committee, School of Biomedical Sciences, College of Health Sciences, Makerere University

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(#SBS 421) and the Health Research Ethics Committee of Stellenbosch University (#S17/09/179). Caregiver provided informed consent for their children/adolescents to participate in the study and for a blood specimen to be drawn for genetics analyses. Adolescents provided further assent to participate in the study. Study participants who were diagnosed with significant psychiatric problems were referred to mental health units at Entebbe and Masaka government hospitals.

2.3.6 Measure of chronic stress

Chronic stress was measured as social disadvantage and variables that were considered to confer social disadvantage were used to construct a composite index for chronic stress. A composite index of chronic stress was constructed from data collected on the following variables: orphanhood (double orphanhood carried a higher chronic stress score vs. single or not orphaned); food availability (not enough food carried a higher chronic stress score vs. enough food); study site (urban carried a higher chronic stress score than rural); and caregiver level of education (no formal education carried a higher chronic stress scores than primary and primary a higher stress score than secondary, etc.). Hierarchical cluster analysis using Statistica 13.5 software (TIBCO, CA, USA), Euclidian distance, as distance measure and Ward's method for clustering (Ward, 1963), was used to generate the different cut-off points for each chronic stress class.

2.3.7 Chronic stress classes

The chronic stress index ranged from 0 to 3.75, with a normal distribution. A total of three chronic stress classes were generated during HCA, i.e., mild, moderate, and severe. The mild class had a chronic stress score of 0 to 1.375, the moderate class had a score of greater than 1.375 to 2.375, and the severe class had a score of greater than 2.375.

2.3.8 Analysis of relative telomere length

DNA was extracted from blood collected from each participant, using the QiAmp Mini DNA Extraction Kit (Qiagen GmbH, Germany). Extracted DNA was quantified by 260/280 and 260/230 ultraviolet spectrophotometry on the NanoDrop 1000 spectrophotometer V3.7 (Thermo Fisher Scientific, Wilmington, MA). The DNA was subsequently diluted to 5 ng/µl and amplified using the KAPA SYBR FAST quantitative polymerase chain reaction (qPCR) Master Mix (Merck, Darmstadt, Germany) per Cawthon *et al*. (2002), with slight modifications

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as a result of PCR optimizations for the primer and DNA concentrations that gave the best amplification plots for the telomere and human β-globin gene (*HBG*) assays respectively (please see Addendum A for the optimized amplification plots for the telomere and *HBG* assays). Primers specific for telomeric repeats (T) (Cawthon, 2002) and a stably expressed single copy reference gene (S), the human β-globin (HBG1, 5′-GCTTCTGACACAACTGTGTTCACTAGC-3′; and HBG2, 5′-CACCAACTTCATCCACGTTCACC-3′), were used to amplify telomeric repeats and *HBG*, respectively. For the telomere assay, each reaction included 5 μl of KAPA SYBR FAST qPCR Master Mix (Merck, Darmstadt, Germany); 1.35 and 4.50 μM of forward and reverse primers, respectively; 5 ng of genomic DNA; and water in a 10-μl reaction volume. The *HBG* assay was identical to the telomere assay except that 2.0 μM of each of the forward and reverse primers were used.

In order to control for interplate variability for a given sample, both the telomeric repeats and the human β-globin gene for that sample were amplified on the same 384-well plate. All samples were randomized at DNA extraction and were plated across the 384-well plate, with the first row containing samples for the telomeric repeats assay and the next row containing the same samples for the human β-globin assay. In order to control for intraplate variability, each participant's DNA sample was amplified in triplicate to reduce measurement variability within samples and this was ensured by checking that Ct values differed by no more than 0.5 across the three wells, and used the mean Ct value in the analysis. If the threshold cycle (Ct) values of the triplicates of particular samples differed by more than 0.5, those samples were excluded. Amplification was performed on the ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA) using the following thermal cycling profile: 95° C for 3 min, followed by 40 cycles of 95° C for 3 s and 60° C for 30 s, and a dissociation stage of 95° C for 15 s, 64° C for 15 s, and 95° C for 15 s. A calibrator sample was prepared by pooling equal amounts of DNA from each participant for the construction of a standard curve.

The calibrator DNA sample was serially diluted 1.68-fold per dilution, to produce a nine-point standard curve, with DNA amounts ranging from 50 to 0.79 ng/μl. After amplification of the standards dilutions, a linear plot of the Ct versus the log value of the input amount of DNA (standard curve) was constructed using ABI's SDS v.2.3 software. The slope of the standard curve was used to calculate the efficiency of the qPCR reaction (efficiency = 10^(-1/slope).

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Standards were repeated across plates in order to control for interplate variability. Threshold and baseline values were used as determined by the SDS v.2.3 software. All Ct values were corrected for the PCR efficiency during the first step of GenEx processing using GenEx software (GenEx User Guide, 2012). GenEx software was also used to control for interplate calibrations.

A validated qPCR method (Cawthon, 2002) was used to determine relative TLs (rTLs) in all samples. First, the mean telomere repeat copy number (tel, T) was normalized to a reference gene (single copy gene) (scg, S) copy number to control for differences in DNA quantity. The T/S ratio is proportional to the average TL. Thereafter, the factor by which the T/S ratio differs between the experimental sample and the calibrator sample is determined to provide an indication of relative average TL:

$T/S = 2^{-\Delta Ct}$

Relative average TL (tel) = $2^{-\Delta\Delta Ct}$

Where $\Delta Ct = Ct(tel) - Ct(scg)$.

A $T/S > 1$ indicates that the average rTL in the sample is greater than that of the reference sample, and a $T/S < 1$ indicates that the average rTL in the experimental sample is less than that in the reference sample.

2.3.9 Power of the study

We calculated the *post hoc* power for our study based on results from a study by Epel *et al.* (2004). We used the formula of sample size and power for difference in means in case–control studies (Kirkwood & Sterne, 2003). We worked on the assumption that cases (individuals with IMDs) would have higher levels of stress than controls (individuals without IMDs). Epel *et al.* (2004) found a 15% reduction in mean rTL among cases compared with controls. Given a 1:1 ratio of cases to controls and using a 5% level of significance, with 368 cases and controls, our study was well powered (power greater than 80%) to detect any reduction above 4.75% in mean rTL between cases and controls. For instance, a reduction of 5% in mean rTL between cases and controls provided a power of 83.8%.

2.3.10 Statistical methods

Statistical analyses were conducted using Stata 15 (StataCorp, TX, USA).

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Socio-demographic characteristics were described between cases and controls. Chi-square tests were used to assess the association between the socio-demographic characteristics and IMDs at baseline (cases vs. controls). SES was generated from a scale of nine household items (car, motorcycle, refrigerator, electricity, bicycle, radio, telephone, cupboard, and flask). Each item was weighted in the respective order, a car carrying a maximum weight of 9 and a flask a minimum weight of 1. A total score of items was generated, whose median cutoff of 13 was used to classify low and high SES. A score less than 13 was classified as low SES, while that greater than 13 was classified as high SES. Our study group (Kinyanda *et al*., 2011a) has previously used household items as a measure of SES in rural settings of Uganda. A *t*-test was used to compare CD4 counts between cases and controls to account for any disparity in HIV disease progression.

Outliers were revealed by box and whisker plots and were all removed from the rTL data. The skewed rTL data became normally distributed after removal of outliers. The distributions of rTL at baseline and at 12 months and the change in rTL were determined using a standardized normal probability plot (P-P plot) (See **Supplementary Materials** in section 2.14 below).

The difference in rTL distribution at baseline and at 12 months was assessed using *t*-tests. The mean difference in rTL between cases and controls was also assessed using *t*-tests. The difference in rTL change between cases and controls was also assessed using *t*-tests. One-way analysis of variance was used to assess whether there were any statistically significant differences between change in rTL and each of the variables of age, sex, study site, caregiver education level, and child education level.

Linear regression was used to i) assess the relationship between rTL and chronic stress, adjusting for sex and age; ii) model the effect of chronic stress on the association between IMDs and rTL, by comparing models without chronic stress to models with chronic stress; and iii) model the effect of age on the relationship between IMDs and rTL. These models (interactions) were performed on all the explanatory variables even without observing significant main effects in order to rule out the possibility of cross over interaction where significant interactions may be observed for non-significant main effects.

There were missing data for rTL values at baseline or 12 months or both. For all analyses that needed computation of confidence intervals, we computed 95% confidence intervals; statistical

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significance was set at a *p*-value less or equal to 0.05, while a *p*-value greater than 0.05 but less than 0.07 was considered a trend towards marginal significance.

2.4 Results

Socio-demographic factors were evenly distributed between cases and controls as shown in **Table 2.1**.

Table 2.1 Distribution of socio-demographic factors of study participants between cases and controls

Variable (n)	Case n (%)	Control n (%)	P-value
Sex			P=0.111
Male (342)	160(46.8)	182(53.2)	
Female (393)	207(52.7)	186(52.7)	
Study site			P=0.941
Urban (415)	208(50.1)	207(49.9)	
Rural (321)	160(49.8)	161(50.2)	
Age			P=0.374
7-11 years (389)	202 (51.9)	187(48.1)	
12-17 years (307)	149(48.5)	158(51.5)	
Education level (Caregiver)			P=0.371
No formal education (13)	9(69.2)	4(30.8)	
Primary (648)	323(49.9)	325(50.1)	
Secondary (35)	35/72(48.6)	37/72(51.4)	
SES			P=0.459
Low (332)	171/332(51.5)	161/332(48.5)	
High (404)	197/404(48.8)	207/404(51.2)	
Mean CD 4 count at baseline	947.04	944.02	P=0.939

CD4, cluster of differentiation 4; primary, 0 – 7 years of formal education; Secondary, 8 – 14 years of formal education; Low SES, 0 – 13; High SES, > 13. All numbers that do not add up were due to missing data.

The standard curves for both the Tel and *HBG* reactions, as well as the dissociation curve for each plate, are shown in addendum E. Tests of association between different socio-demographic variables and rTL were run to determine potential confounders (**Table 2.2**). None of the socio-demographic variables were associated with rTL (**Table 2.2a**). Study site, age, and

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SES were significantly associated with chronic stress ($p < 0.001$, p = 0.040, and p = 0.015, respectively) (**Table 2.2b**).

Table 2.2a P-values for tests of association between socio-demographic variables and rTL change

Variable	Category	Mean rTL	P-value
Sex	Male	0.270	0.298
	Female	0.266	
Study site	Rural	0.221	0.235
	Urban	0.271	
Age of child/adolescent	5 – 8	0.232	0.553
	9 – 12	0.276	
	13 – 17	0.225	
Caregiver level of education	None	0.161	0.912
	Primary	0.143	
	Secondary	0.266	
	Tertiary	0.244	
	Other	0.295	
Socio-economic status	Low	0.248	0.897
	High	0.243	
Child/adolescent education level	No formal	0.387	0.611
	Pre-primary	0.243	
	Secondary	0.229	

Table 2.2b P-values for tests of association between socio-demographic variables and chronic stress

Variable	Category	Number in each chronic stress class			P-value
		Mild	Moderate	Severe	
Sex	Male	110	156	76	0.122
	Female	153	169	71	
Study site	Rural	235	75	11	<0.001
	Urban	29	250	136	
Age of child/adolescent	5 – 8	114	107	125	0.040
	9 – 12	83	125	51	
	13 – 17	67	93	50	
Caregiver education level	None	17	15	1	0.587
	Primary	24	11	6	
	Secondary	60	124	58	
	Tertiary	146	139	55	
	Other tertiary	11	29	25	
Socio-economic status	Low	127	128	77	0.015
	High	137	197	70	
Child/adolescent education level	No formal	8	3	2	0.364

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	Pre-primary	232	287	129
	Secondary	23	34	15

2.4.1 Difference in rTL between cases and controls

RTL was normally distributed at both baseline and 12 months. Mean rTL (95%CI) of the combined sample of cases and controls was 1.148 (1.119–1.176) at baseline and 0.905 (0.879–0.931) at 12 months. For cases, mean rTL (95%CI) was 1.198 (1.157–1.239) at baseline and 0.925 (0.886–0.965) at 12 months; while for controls, mean rTL (95%CI) was 1.097 (1.057–1.137) at baseline and 0.884 (0.851–0.917) at 12 months.

At baseline, we found a statistically significant difference in rTL between cases and controls ($p < 0.001$). However, contrary to what we expected, rTL was longer in cases compared with controls. There was, however, no statistical difference in rTL between cases and controls at 12 months (p = 0.117). In addition, the change between baseline and 12-month rTL (rTL change) did not differ statistically between cases and controls (p = 0.608) (**Table 2.3**).

Table 2.3 Difference in rTL between cases and controls

Time point	Group	Observations	Mean rTL	Std. Dev	95% CI	P-value
Baseline	Cases	307	1.19807	0.36415	1.15717 - 1.23896	<0.001
	Controls	306	1.09668	0.35382	1.05688 - 1.13648	
12 month	Cases	278	0.92517	0.33559	0.88555 - 0.96480	0.117
	Controls	274	0.88419	0.27484	0.85150 - 0.91687	
rTL change	Cases	231	-0.25560	0.47261	0.19434 – 0.31688	0.608
	Controls	234	-0.23444	0.41546	0.18093 – 0.28795	

2.4.2 Differences between baseline and 12-month rTL (Change in rTL)

In the combined analysis of baseline cases and controls, there was significant attrition in rTL between baseline and 12 months (p < 0.001). This attrition did not differ by IMD status (p = 0.608). A further stratified analysis of cases only and controls only yielded similar p-values of <0.001 (**Table 2.4**).

Table 2.4 Differences between baseline and 12 months rTL for baseline cases and controls

Group	Time point	Obs	Mean TL	Std. Dev	95% Conf. Interval	P-Value
Total sample	Baseline	465	1.14827	0.36557	1.11495 - 1.18158	p<0.001

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	12 month	465	0.90331	0.31397	0.87470 - 0.93192	
Cases	Baseline	231	1.19006	0.37096	1.14197 - 1.23815	
	12 month	231	0.93445	0.34778	0.88937 - 0.97954	p<0.001
Controls	Baseline	234	1.10701	0.35618	1.06113 - 1.15288	
	12 month	234	0.87257	0.27387	0.83730 - 0.90784	p<0.001

2.4.3 Association between chronic stress and rTL

We observed a trend towards statistical significance between chronic stress and baseline rTL (p = 0.067). Severe stress was significantly associated with longer rTL (p = 0.028) (**Table 2.5**). However, chronic stress was not significantly associated with either 12-month rTL or a change in rTL (p = 0.147 and p = 0.455, respectively) (**Table 2.5**).

Table 2.5 Assessing association between chronic stress and rTL, adjusted for age and sex

Time point	Chronic stress class	Coefficient	P>|t|	95% Conf. Interval	P-value
	Mild	Reference			
	Moderate	0.01251	0.717	-0.05514 - 0.08017	
Baseline	Severe	0.09144	0.028	0.00991 - 0.17298	0.067
	Mild	Reference			
	Moderate	-0.03123	0.288	-0.08887 - 0.02640	
12 month	Severe	0.03416	0.334	-0.03520 - 0.10353	0.147
	Mild	Reference			
	Moderate	0.05759	0.214	-0.03339 - 0.14858	
rTL change	Severe	0.03707	0.507	-0.07254 - 0.14667	0.451

Reference, reference chronic stress class during regression analysis.

2.4.4 Association between chronic stress and IMDs

We found a trend toward statistical significance between chronic stress and IMDs (**Table 2.6**).

Table 2.6 Association between chronic stress and Internalizing mental disorders

Chronic stress class	Cases (n)	Controls (n)	Total	p-value
Mild	120	144	264	
Moderate	178	147	325	
Severe	70	77	147	
Total	368	368	736	0.065

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2.4.5 The rTL and IMDs after 12 months

We found no significant difference in baseline rTL between cases of IMDs that persisted compared to those that remitted after 12 months (p = 0.235). We also found no statistically significant association between 12-month rTL and 12-month IMD status (*p* = 0.090), as well as no association between disease severity and rTL at baseline (p = 0.238) and 12 months (p = 0.264).

2.4.6 Effect of chronic stress on the association between IMDs and rTL

We found no significant influence of chronic stress on the association between IMDs and rTL both at baseline and at 12 months (**Table 2.7**).

Table 2.7 Two-way analysis of variance for the interaction of chronic stress with IMDs on baseline and 12 months TL

Outcome	Observations	Variable	F	P-value
Baseline TL	613	IMDs	11.72	<0.001
		Chronic stress	2.48	0.085
		IMDs * chronic stress	0.14	0.870
12 months TL	552	IMDs	2.31	0.130
		rs2736100	1.71	0.182
		IMDs * rs2736100	0.57	0.566

* = interaction term.

2.4.7 Effect of age on the relationship between IMDs and rTL

On stratifying our analyses for age [children (7–11 years) and adolescents (12–17 years)], we observed no statistically significant differences by age group for IMDs and rTL compared with those that were observed with both age categories combined (**Table 2.8**).

Table 2.8 Effect of age on the association between IMDs and rTL

Baseline					
Category	IMDs status	Coefficient	P>|t|	95% Conf. Interval	P-value
Total sample	Controls	Reference			
	Cases	0.101	0.001	0.04443 - 0.15834	P<0.001
Children	Controls	Reference			
	Cases	0.094	0.025	0.01203 - 0.17534	0.025
	Controls	Reference			

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Adolescents	Cases	0.110	0.007	0.03080 - 0.18999	0.007
12 months					
Category	IMDs status	Coefficient	P>|t|	95% Conf. Interval	P-value
Total sample	Controls	Reference			
	Cases	0.041	0.117	-0.01034 - 0.09231	0.117
Children	Controls	Reference			
	Cases	0.048	0.181	-0.02234 - 0.11776	0.181
Adolescents	Controls	Reference			
	Cases	0.033	0.395	-0.04295 - 0.10858	0.395
rTL change					
Category	IMDs status	Coefficient	P>|t|	95% Conf. Interval	P-value
Total sample	Controls	Reference			
	Cases	0.021	0.608	-0.05990 - 0.10223	0.601
Children	Controls	Reference			
	Cases	0.017	0.768	-0.09841 - 0.13321	0.768
Adolescents	Controls	Reference			
	Cases	0.027	0.639	-0.08634 - 0.14043	0.639

Children, 7-11 years; Adolescents, 12-17 years; reference, reference IMDs status during regression analysis.

2.5 Discussion

In this study, we investigated the association between chronic stress and rTL among PHIY cases with IMDs and age- and sex-matched controls in Uganda. To our knowledge, this is the first sub-Saharan African study to investigate the association between chronic stress with rTL and IMDs among PHIY.

Several studies have determined the association between TL and different internalizing psychopathologies. Shorter TL have been reported among cases of depression compared with controls (Garcia-Rizo *et al*., 2013; Shalev *et al*., 2014; Verhoeven *et al*., 2014), while others have failed to find significant associations (Wolkowitz *et al*., 2011a; Teyssier *et al*., 2012; Simon *et al*., 2015). Shorter TL has also been implicated in both anxiety disorders (Kananen *et al*., 2010; Verhoeven *et al*., 2015) and PTSD (O'Donovan *et al*., 2011; Zhang *et al*., 2014) and has been reported to confer risk for PTSD (Malan *et al*., 2011). Due to these reported associations of shorter TL in the different internalizing psychopathologies, we hypothesized that rTL would be shorter among cases of IMDs than controls in our study participants. Contrary to our hypothesis, we observed longer rTL among cases of IMDs compared with their controls ($p < 0.001$). Longer rTL among IMDs could be due to elevated telomerase levels. TL is maintained by a telomerase enzyme component known as telomerase RNA component (TERC) and a reverse transcriptase enzyme known as the telomerase reverse transcriptase

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(TERT) (Wang and Meier, 2004; Blackburn, Geider & Szostak, 2006). Wolkowitz *et al*. (2012) indeed reported elevated telomerase levels among people with depression than among healthy matched controls at baseline. After 8 weeks of treatment with selective serotonin re-uptake inhibitors, they found that telomerase levels became even more elevated as depression remitted. It has been speculated that elevated telomerase levels are a compensatory effort towards excessive loss of telomeres (Damjanovic *et al*., 2007; Lin *et al*., 2012).

We also observed a statistically significant reduction in rTL between baseline and 12 months in a combined sample of cases and controls ($p < 0.001$). A statistical difference was also observed when cases and controls were individually analyzed ($p < 0.001$). This difference was expected since TL generally decreases over the life span (Müezzinler, Zaineddin & Brenner, 2013). We found no significant difference in rTL between cases and controls at 12 months ($p = 0.117$). Since cases had significantly longer rTL than controls at baseline ($p < 0.001$), the lack of a significant difference at 12 months indicates greater rTL attrition among cases compared with controls. This is an interesting observation that points to the notion that IMDs are possibly driving accelerated cellular aging (rTL attrition). Indeed, telomere shortening has been reported to be strongly influenced by chronic stress exposure (Ridout *et al*., 2015), and suffering from a chronic disease, such as heart disease (Haycock *et al*., 2014) and diabetes (Zhao *et al*., 2013), has been conceptualized as a prolonged stress exposure that could explain their association with TL. IMDs have been reported as chronic stressors (McEwen, 2003) with chronic biological adaptations that result in long-term biological damage that could potentially explain rTL attrition due to IMDs (Epel & Lithgow, 2014).

IMDs could also be leading to rTL attrition through inflammatory pathways. Depression has been reported to prime larger cytokine responses to stressors (Kiecolt-Glaser, Derry & Fagundes, 2015). Increased systemic inflammation has been associated with decreased TL among a prospective cohort of workers exposed to high level of fine particulate matter (Wong *et al*., 2014), while interventions that attenuate inflammatory processes in fear- and anxiety-based disorders have been thought to be effective in mitigating the symptoms of anxiety disorders (Michopoulos *et al*., 2017).

If IMDs were driving rTL attrition, we would expect significant reduction in rTL among cases with no corresponding significant reduction among controls. Intriguingly, we observed significant reduction in rTL in both groups ($p < 0.001$). This is possibly due to general reduction

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in rTL that occurs over time. However, study subjects were only followed up for 12 months, and a longer follow-up period may be required to see a true difference in rTL attrition between cases and controls and thus more data is needed to justify our hypothesis. A number of factors affect TL, many of which may have been important to account for in the present study. For example, participants were all on ART, with the type and duration of ART regimen not accounted for in the analysis, yet total time receiving ART and duration of treatment with nucleoside reverse transcriptase inhibitors have been associated with shorter telomere length (Montejano *et al*., 2017). In addition, factors known to affect rTL, such as diet (Shiels *et al*., 2011) and frequency of physical exercise (Cherkas *et al*., 2008) were not accounted for. Moreover, studies have found that the effects on rTL may be determined even before birth from maternal stress, or through direct transmission of maternal rTL (Epel & Pranther, 2018). Also, since HIV infection has been associated with accelerated TL shortening (Alejos *et al*., 2019; Shiau *et al*., 2018; Auld *et al*., 2016), it is possible that effects of HIV and its associated co-infections may have strongly confounded our findings.

Although previous studies among children have found associations between TL and socio-demographic variables, such as caregiver level of education (Needham *et al*., 2012), parental SES (Needham *et al*., 2012; Mitchell *et al*., 2014), sex (Drury *et al*., 2014), and living environments (Theall *et al*., 2013), we found no association between any baseline socio-demographic variables and rTL change in the present study. This discrepancy could be due to cultural context, as previous studies were carried out in developed world settings that differ from the African low-income setting of this study. For example, stress due to orphanhood in the Ugandan context may be experienced differently, as there is a strong extended family system in Uganda where orphans tend to be taken care of by their uncles or aunts, unlike in the developed world where orphans are often institutionalized. The latter has been associated with shorter TL (Drury *et al*., 2012). More studies are needed to understand factors that affect TL in the sub- Saharan African context.

We found no association between rTL change and persistence or remission of IMDs. This further suggests that rTL does not drive IMDs, but rather IMDs may be driving accelerated cellular aging. Higher mortality rates have been reported among patients with IMDs compared with the general population, and the mortality is mainly due to the same age-related diseases as the general population, such as cancer, and heart, and cerebrovascular disease. For example, a study by Colton and Manderscheid (2006) reported that clients with a diagnosis of major

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mental illness died 1 to 10 years earlier than did clients with no major mental illness. Another study reported that persons with mental disorders died an average of 8.2 years younger than did the rest of the population and that presence of a mental illness was associated with a hazard ratio of 2 over a 17-year study period (Druss *et al*., 2011), supporting the mediating role of IMDs in accelerated cellular aging.

Psychological stress (both perceived stress and chronicity of stress) has been significantly associated with lower telomerase activity and shorter TL (Epel *et al*., 2004). We investigated the association between chronic stress and rTL in our sample. We observed a marginally significant association between chronic stress and rTL ($p = 0.067$). However, contrary to expectation, severe chronic stress was associated with longer rTL ($p = 0.028$) (**Table 2.5**). Longer rTL was also associated with IMD caseness. Thus, if increased stress (chronic) is an acquired vulnerability factor for IMDs, then it stands to reason that severe chronic stress would be associated with longer rTL, an association that we indeed observed. Further, since IMDs are associated with impaired quality of life and negative clinical and behavioral outcomes among PHIY and poor adherence to ART (Malee *et al*., 2011; Walkup *et al*., 2009), we expected significantly lower CD4 counts among cases than controls. However, we found no significant difference in mean CD4 count between cases and controls ($p = 0.939$). We did not investigate other virologic markers of HIV disease severity, such as viral load. However, all study participants were on ART, and thus no difference would be expected if adherence to treatment was similar between cases and controls.

We observed an association between chronic stress and study site and SES respectively. Living in urban areas and having a high SES were associated with more chronic stress than living in rural areas and having a low SES. The association of both urban location and high SES with increased chronic stress may be due to a correlation between the two variables, as participants in urban areas are often of higher SES as compared with their rural counterparts. The association of urban location with increased chronic stress could be due to ecological factors and pressures that are associated with urban life as compared with rural life.

We also observed an association between age and chronic stress. Adolescents (12–17 years) were more stressed than children (7–11 years) and this could be due to the fact that adolescents were aware of their HIV status and the stress could be associated with the burden of being HIV+ and stigma among these study participants (Knizek *et al*., 2017).

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Lastly, since severe chronic stress is associated with longer rTL, we expected severe chronic stress to lower the p-value of the regression for the association between IMDs and rTL, an interaction we did not observe. We think that this could due to duration of chronic stress. Although the chronic stress variables used in the present study are known stressors in this population, the duration of the stressor was not assessed for.

2.6 Limitations and recommendations

We defined IMDs as having any depressive disorder or anxiety disorder or PTSD. The inclusion of PTSD as an IMD is contentious as the disorder has recently been delineated from IMDs in the DSM-5. PTSD has been associated with the most robust TL attrition (Darrow *et al*., 2016), it is possible that the TL differences observed between baseline and 12 months may have been strongly influenced by inclusion of PTSD. We recommend that future studies undertake a comparative analysis of the different disorders that make up the IMD spectrum to elucidate the independent contribution of each particular disorder.

Due to lack of data, we did not investigate factors that are known to affect rTL, such as frequency of physical activity, medication, diet, and presence of other comorbid diseases. Also, much as CD4 counts did not significantly differ between cases and controls, the ART regimen for each study participant was not accounted for in the analysis. Future studies should endeavor to consider these factors.

Both the duration and severity of IMDs have been shown to affect rTL. We did not assess the duration of IMDs. However, we think that this may not have greatly affected our findings because disease severity was not significantly associated with rTL in the present study. Future studies should, however, account for the duration of IMDs.

We suggest that the significantly longer rTL observed among cases at baseline is due to elevated telomerase activity/levels. However, we did not investigate telomerase activity/levels between cases and controls. Future studies should investigate this possibility. Also, certain genes, such as the telomerase reverse transcriptase and telomerase RNA component, have been reported to influence TL biology. The role of polymorphisms in these genes influencing rTL needs to be investigated, and future studies should endeavor to address this.

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The qPCR efficiencies were outside the optimal range of 90 – 110% (Čepin, 2017) (please see addendum E for the standard curves and the dissociation graphs). The possible reasons for this observation have been discussed under section 5.7 of this PhD thesis.

Chronic stress was measured using a number of context-specific indicators because there is no locally adapted tool for assessing chronic stress in this setting. While this may be a limitation and may limit generalizability to other settings, the variables used to generate the chronic stress index are known stressors in this population. Validation of the chronic stress index tool will be required in future studies in Uganda.

2.7 Conclusions

RTL was longer in cases with IMDs compared with age- and sex-matched controls.
We observed significant attrition in rTL over 12 months. This rTL attrition seems to be driven by the presence of any IMDs, indicating that IMDs could be driving accelerated rTL attrition. Mechanisms that either directly influence rTL or alleviate the effects of IMDs on rTL attrition could explain our study findings, and longitudinal and experimental studies are needed to fully elucidate underlying mechanisms.

2.8 Ethics approval and consent to participate

The study obtained ethics approval from the Health Research Committee of Stellenbosch University (#S17/09/179) and the higher Degrees Research & Ethics Committee of the School of Biomedical Sciences, College of Health Sciences, Makerere University (#SBS 421). The parent study (CHAKA) obtained ethics approval from the Uganda Virus Research Institute's Science and Ethical Committee (#GC/127/15/06/459) and the Uganda National Council of Science and Technology (#HS 1601). All caregivers provided informed consent for their children/adolescents to participate in the study and for a blood specimen to be withdrawn from them (child/adolescent) for rTL and other genetics analyses in accordance with the Declaration of Helsinki. Adolescents further provided informed assent to participate in the study.

2.9 Consent for publication

No details, images, or videos relating to any of the study participants are included in this manuscript.

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2.10 Author contributions

Concept was provided by AK, SMJH, EK, and SS. Data collection was done by AK, EK, SMJH, JSW, and SS. Data analysis was done by WS, AK, RNN, SMJH, JSW, SS, MK, and JL. First draft was done by AK, SMJH, JSW, WS, EK, SS, MLJ, RNN, PK, MK, and JL. Final revision was done by AK, SMJH, JSW, EK, SS, WS, MLJ, RNN, PK, MK, and JL. All authors read and approved the final manuscript.

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2.13 Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.14 Supplementary materials

Probability-probability (p-p) plots showing the distribution of rTL at baseline and 12 months, and the change in rTL. The p-p plots were used to assess for normality of the rTL data, the closer the data points are to the diagonal line, the closer to normal distribution the data is.

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Baseline (Figure 2.1) and 12 month rTL (Figure 2.2), as well as the rTL change (Figure 2.3), were normally distributed as revealed by the plots below.

Figure 2.1: p-p plot for distribution of TL at baseline

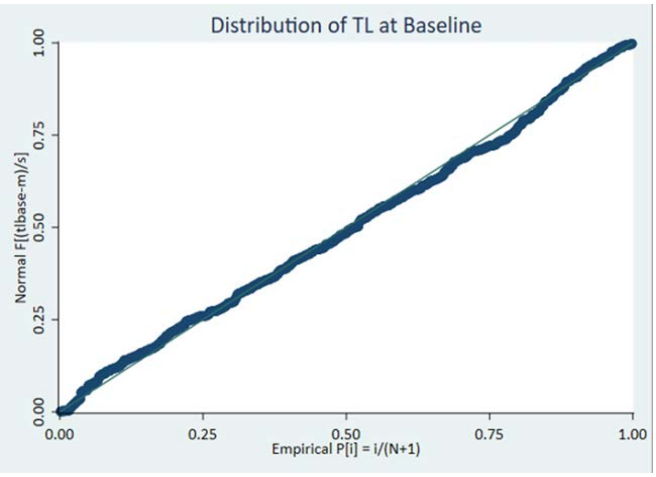


Figure 2.2: p-p plot for distribution of TL at 12 months

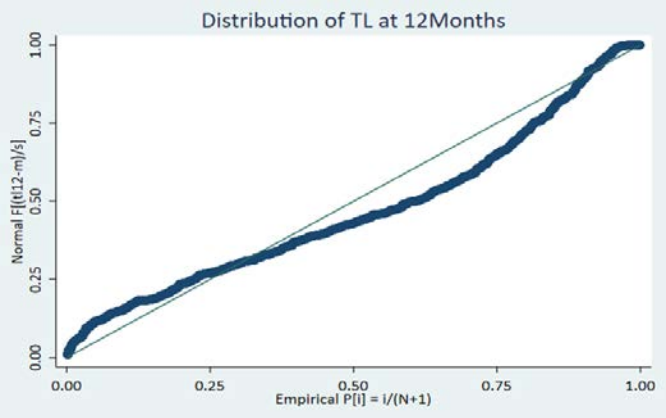
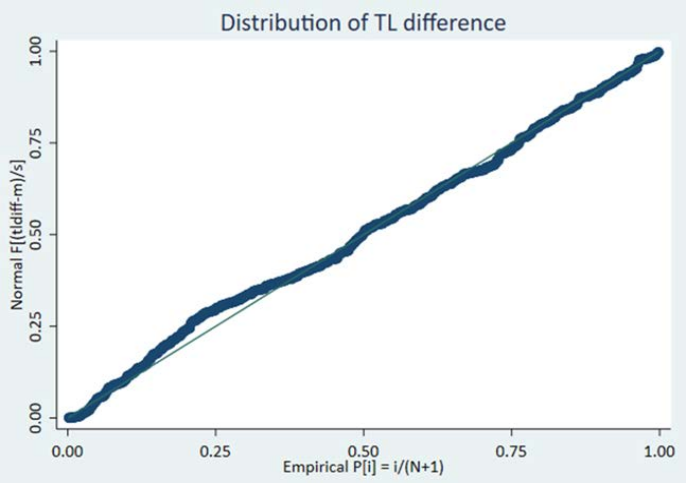


Figure 2.3: p-p plot for distribution of the difference between baseline and 12 months TL



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CHAPTER THREE

This chapter represents a manuscript that highlights the moderating role of genetic variants in *TERT* and *TERC* on the association between IMDs and accelerated TL attrition among the study participants. This manuscript has been submitted to *BMC Medical Genetics (Impact factor 1.913)* for publication and is currently under editorial assessment.

The telomerase reverse transcriptase gene rs2736100 and telomerase RNA component gene rs16847897 moderate the association between internalizing mental disorders and accelerated telomere length attrition among HIV+ children and adolescents in Uganda

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3.1 Abstract

Background

Internalizing mental disorders (IMDs) (depression, anxiety and post-traumatic stress disorder) have been associated with accelerated telomere length (TL) attrition; however, this association has not been investigated in the context of genetic variation that has been found to influence TL. We have previously reported an association between IMDs and accelerated TL attrition among Ugandan HIV+ children and adolescents. This study investigated the moderating effects of selected single nucleotide polymorphisms in the telomerase reverse transcriptase gene (*TERT*) (rs2736100, rs7726159, rs10069690 and rs2853669) and the telomerase RNA component gene (*TERC*) (rs12696304, rs16847897 and rs10936599) on the association between IMDs and TL, among Ugandan HIV+ children (aged 5–11 years) and adolescents (aged 12–17 years).

Results

We found no significant interaction between IMDs and any of the selected single nucleotide polymorphisms (SNPs) on TL at baseline. We observed significant interactions of IMDs with *TERT* rs2736100 ($p = 0.007$) and *TERC* rs16847897 ($p = 0.012$), respectively on TL at 12 months.

Conclusions

TERT rs2736100 and *TERC* rs16847897 moderate the association between IMDs and TL among Ugandan HIV+ children and adolescents at 12 months. Understanding the nature of this association may shed light on the pathophysiological mechanisms underlying advanced cellular aging in IMDs.

Key words: Internalizing mental disorders, telomere length attrition, *TERT* rs2736100, *TERC* rs16847897, HIV+ children and adolescents, Uganda

3.2 Background

Human immunodeficiency virus-positive (HIV+) children and adolescents suffer a considerable burden of internalizing mental disorders (IMDs) (namely, depression, anxiety and posttraumatic stress disorder) (Kinyanda *et al.*, 2019; Mellins *et al.*, 2012; Nachman *et al.*, 2012). Studies undertaken both in the developed (Europe and the United States) and

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developing world (sub-Saharan Africa) among HIV+ children and adolescents have documented rates of major depressive disorder of between 12.7% and 40% (Mellins *et al*., 2012; Nachman *et al*., 2012; Lwidiko *et al*., 2018; Kim *et al*., 2014; Kamau *et al*., 2012; Gadow *et al*., 2012; Musisi & Kinyanda, 2009) and rates of anxiety disorders of between 9% and 32.2% (Kinyanda *et al*., 2019; Mellins *et al*., 2012; Nachman *et al*., 2012; Kamau *et al*., 2012). For IMDs combined, rates of between 12% and 27% have been documented in Uganda and South Africa, respectively (Kinyanda *et al*., 2019; Woollet, Cluver, Bandeira & Brahmbhatt, 2017). Among people living with HIV/AIDS, IMDs have been associated with a number of other negative outcomes, including accelerated cellular aging (Kalungi *et al*., 2019), faster HIV disease progression (Chida & Vedhara, 2009; Ironson *et al*., 2005), poor adherence to medication (Ironson *et al*., 2005; Kinyanda *et al*., 2018), risky sexual behavior (Kinyanda *et al*., 2018; Springer, Dushaj & Azar, 2012), poor linkage to care for newly diagnosed HIV+ persons (Bhatia *et al*., 2011), increased HIV transmission (through the promotion of HIV drug resistance) (Remien & Mellins, 2007) and impaired academic and social functioning (Kinyanda *et al*., 2018).

While considerable research has investigated the psychosocial risk factors for IMDs among HIV+ children and adolescents, there is a paucity of research on biological risk factors, including genetic factors. Recent GWASes have identified loci for depression (Wray *et al*., 2018), anxiety disorders (Meier *et al*., 2019) and PTSD (Nievergelt *et al*., 2019; Gelernter *et al*., 2019), providing evidence for the role of genetic variation in the etiology of IMDs. Nevertheless, the underlying etiology, and biochemical and molecular events in particular, of IMDs are largely unknown.

IMDs are associated with increased mortality and age-related disease such as cancer, heart and cardio-vascular disease (Pratt, Druss, Manderscheid & Walker, 2016; Druss *et al*., 2011; Colton & Manderscheid, 2006; Cuijpers & Smit, 2002). IMDs are also highly comorbid with both psychiatric and somatic disorders, including those associated with advanced aging (Lotfaliany *et al*., 2019). Depression has, for example, been reported to be associated with chronic diseases (e.g., type 2 diabetes mellitus and cardiovascular disease) (Lotfaliany *et al*., 2019; Mason, Beck & Muruwe, 2012), as well chronic inflammation (Leonard, 2007). In addition, higher mortality rates have been reported among patients with mental disorders (e.g., depression and other affective disorders) compared to the general population, with this mortality mainly due to the same age-related diseases, such as cancer, cardiac and cerebrovascular disease (Colton &

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Manderscheid, 2006; Cuijpers & Smit, 2002; Druss *et al*., 2011). Evidence for the role of TL among patients with IMDs and comorbid age-related diseases may offer a novel potential mechanism for the excess morbidity and mortality associated with IMDs (Ramunas *et al*., 2015).

Several studies have investigated the association between telomeres, the protein-bound deoxyribonucleic acid (DNA) repeat structures at the ends of chromosomes, and IMDs (Lindqvist *et al*., 2015). Telomeres are important in protecting chromosomes from fusing together during mitosis, thus preventing loss of genetic data (Allsopp *et al*., 1992; Blackburn, Geider & Szostak, 2006). They shorten progressively with each cell division, eventually leading to DNA damage responses, replicative senescence, or programmed cell death (Zlotorynski, 2019). Since telomeres shorten with each cycle of cell division, TL provides a marker of biological aging (Shay, 2016).

Shorter TL has been reported among patients with depression (Verhoeven *et al*., 2017; Lin, Huang & Hung, 2016; Schutte & Maouff, 2015; Garcia-Rizo *et al*., 2013; Verhoeven *et al*., 2014; Shalev *et al*., 2014; Douillard-Guilloux, Guiolloux, Lewis & Sibille, 2013; Kinser & Lyon, 2013), anxiety disorders (Verhoeven *et al*., 2017; Verhoeven *et al*., 2015; Kananen *et al*., 2010) and PTSD (Avetyan, Zakharyan, Petrek & Arakelyan, 2019; Roberts *et al*., 2017; O'Donovan *et al*., 2011; Zhang *et al*., 2014).

We previously found evidence to suggest accelerated TL attrition that was driven by IMDs among Ugandan HIV+ children and adolescents (Kalungi *et al*., 2019). There is, however, a dearth of data on the mechanisms by which IMDs lead to TL attrition. IMDs act as chronic stressors (Dieleman *et al*., 2015; McEwen, 2003), producing long-lasting biological adaptations that could potentially explain TL attrition due to IMDs (Epel & Lithgow, 2014). Specifically, chronic stress can exert long-lasting effects on the hypothalamic-pituitary-adrenal axis, such that previous experience of stress may prime heightened response on subsequent stressor exposure (Aristizabal *et al*., 2019). Chronic stress also increases inflammatory signaling, which may in turn produce a pro-oxidative environment. Both inflammation and oxidative stress have been negatively associated with TL (Kiecolt-Glaser, Derry & Fagundes, 2015).

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TL is heritable, with heritability estimates ranging between 44–80 % (Njajou *et al*., 2007; Broer *et al*., 2013). Therefore, genetic variation may contribute to telomere maintenance (Codd *et al*., 2013) and this genetic variation may confer risk for accelerated TL attrition. TL is maintained by telomerase, a catalytic enzyme with a protein component encoded by telomerase reverse transcriptase gene (*TERT*) and an RNA template component encoded by telomerase RNA component gene (*TERC*). Together, these act to add small DNA repeat segments to the end of chromosomes, thus counteracting TL attrition (Blackburn, Geider & Szostak, 2006; Wang & Meier., 2004). A large genome-wide meta-analysis of 37,684 individuals found that *TERT* and *TERC* were amongst several loci found to influence mean TL (Codd *et al*., 2013), suggesting that genetic variation in these telomerase components has functional implications on TL.

The *TERC* rs12696304 *G*-allele and rs16847897 *C*-allele have been found to be associated with shorter TL by a candidate gene study on Swedish samples and a GWAS on British samples (Codd *et al*., 2010; Melin, Nordfjäll, Andersson & Roos, 2012). In addition, *TERT* rs2736100 *C*-allele has been associated with shorter TL (Codd *et al*., 2013), while *TERT* rs7726159 *AA* genotype was associated with longer TL by a candidate gene study using White European samples (Rode, Nordestgaard & Bojesen, 2016).

We have previously determined an association between IMDs and TL in a sample of Ugandan HIV+ children and adolescents (Kalungi *et al*., 2019). Specifically, TL was significantly longer in IMD cases at baseline but did not differ from control TL at 12-month follow-up, suggesting that TL shortening over the one-year period was greater in participants with IMDs. Given that *TERT* and *TERC* are involved in TL maintenance (Blackburn, Geider & Szostak, 2006), the present study investigated whether genetic variation in *TERT* (rs2736100, rs7726159, rs10069690 and rs2853669) and *TERC* (rs12696304, rs16847897 and rs10936599) moderated the association between IMDs and each of baseline and 12-month TL. Having observed that IMDs were driving accelerated TL attrition in the previous study (Kalungi *et al*., 2019), we modelled IMDs as the independent variable and TL as the dependent variable. We thus assessed for the interaction between IMDs and *TERT* and *TERC* on TL (see statistical methods section).

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3.3 Results

No significant differences were observed when socio-demographic variables were compared between case and control participants (Table 3.1).

Table 3.1: Distribution of socio-demographic factors in cases and controls

Variable (n)	Case n (%)	Control n (%)	P-value
Sex			p = 0.111
Male (342)	160 (43.6)	182 (49.5)	
Female (393)	207 (56.4)	186 (50.5)	
Site			p = 0.941
Urban (415)	208 (56.5)	207 (56.3)	
Rural (321)	160 (43.5)	161 (43.7)	
Age			p = 0.374
5–11 years (389)	202 (57.6)	187 (54.2)	
12–17 years (307)	149 (42.4)	158 (45.8)	
Education level			p = 0.371
No formal education (13)	9 (2.5)	4 (1.1)	
Primary (648)	323 (88.0)	325 (88.8)	
Secondary (72)	35 (9.5)	37 (10.1)	
Socioeconomic status			p = 0.459
Low (332)	171 (46.5)	161 (43.8)	
High (404)	197 (53.5)	207 (56.2)	
Mean CD4 count at baseline			
	947.04	944.02	p = 0.939

CD4 = cluster of differentiation 4; primary = 0 – 7 years of formal education; Secondary = 8 – 14 years of formal education; Low socioeconomic status = 0 – 13; High socioeconomic status = > 13 (see statistical methods section). All numbers that do not add up were due to missing data

The genotypes for all the selected SNPs were in Hardy-Weinberg equilibrium (HWE) (Table 3.2). *TERT* rs7726159 and rs2736100 were in linkage disequilibrium (LD), resulting in *CT*, *CG* and *AG* haplotypes while none of *TERC* SNPs were in LD (see Supplementary materials S3.1 and S3.2). The association between each investigated SNP and TL are shown in Table 3.2 below. None of the selected SNPs and *TERT* rs7726159-rs2736100 haplotypes associated with

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baseline TL or TL change; however, *TERC* rs16847897 significantly associated with 12-month TL (Table 3.2).

Table 3.2: P-values for Hardy-Weinberg equilibrium and association of each selected single nucleotide polymorphism with TL

Single nucleotide polymorphism	P-value of association with			HWE p-value
	Baseline TL	12 months TL	TL change	
TERC rs16847897	0.650	0.014	0.393	0.461
TERC rs12696304	0.861	0.991	0.981	0.443
TERC rs10936599	0.406	0.142	0.510	1
TERT rs2736100	0.300	0.366	0.581	0.091
TERT rs2853669	0.438	0.507	0.591	0.798
TERT rs7726159	0.590	0.855	0.802	0.882
TERT rs10069690	0.567	0.831	0.802	0.274
TERT rs7726159-rs2736100	0.331	0.724	0.725	N/A

HWE = Hardy-Weinberg equilibrium, *TERC* = telomerase RNA component gene, *TERT* = telomerase reverse transcriptase gene, *TERT* rs7726159-rs2736100 = *TERT* rs7726159 and rs2736100 haplotype, N/A = not applicable.

Moderating effects of single nucleotide polymorphisms in telomerase reverse transcriptase gene and telomerase RNA component gene on the association between internalizing mental disorders and telomere length

None of the selected SNPs were found to moderate the association between IMDs and TL at baseline (Table 3.3). We however found that *TERT* rs2736100 and *TERC* rs16847897 significantly moderated the association between IMDs and TL at 12 months ($p = 0.007$ and $p = 0.012$ respectively) (Table 3.3). For *TERT* rs2736100, mean TL was longer in cases compared to controls, but only in those individuals with the *GG* genotype (n = 139; 82 cases, 57 controls) (Figure 3.1). No significant difference in TL was observed between cases and controls who possessed either the *TG* (n = 330) or *TT* (n = 201) genotypes (Figure 3.1). For *TERC* rs16847897, mean TL was longer in cases compared to controls, but only in individuals with the *CC* genotype (n = 44; 27 cases, 17 controls) (Figure 3.1). No significant difference in TL was observed between cases and controls who possessed either the *GC* (n = 265) or *GG* (n = 373) genotypes (Figure 3.1). The results demonstrated that faster TL attrition occurred among

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participants with at least one *T*-allele for *TERT* rs2736100 and at least one *G*-allele for *TERC* rs16847897. P-values for the interaction between the rest of the SNPs and IMDs on baseline TL, 12 months TL and TL change are shown in Table S3.1 (see supplementary materials).

Table 3.3: Two-way analysis of variance for the interaction of IMDs with rs2736100 and with rs16847897 on TL at 12 months

SNP	Obs	Variable	F	Bonferroni corrected P-value (α = 0.025)
Baseline				
rs2736100	596	IMDs	15.52	<0.001
		rs2736100	0.98	0.375
		IMDs * rs2736100	1.15	0.318
rs16847897	597	IMDs	2.53	0.112
		rs16847897	0.37	0.692
		IMDs * rs16847897	1.79	0.168
12 months				
rs2736100	511	IMDs	6.03	0.014
		rs2736100	0.44	0.645
		IMDs * rs2736100	4.95	0.007
rs16847897	515	IMDs	7.25	0.007
		rs16847897	3.00	0.050
		IMDs * rs16847897	4.44	0.012

SNP: single nucleotide polymorphism; Obs: number of observations; IMDs*rs2736100: interaction of internalizing mental disorders with *TERT* rs2736100 on relative telomere length; IMDs **TERC* rs16847897, interaction of internalizing mental disorders with rs16847897 on relative telomere length.

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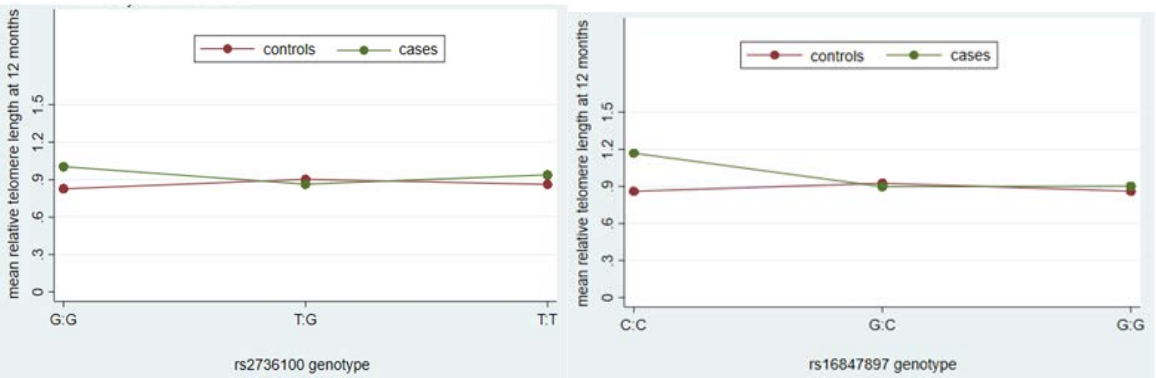

Figure 3.1: Mean TL between cases and controls by genotype for *TERT* rs2736100 and *TERC* rs16847897 at 12 months.

For TERT rs2736100 mean TL was significantly different between cases and controls, but only in individuals with the GG genotype while for TERC rs16847897, mean TL was significantly different between cases and controls but only in individuals with CC genotype.

3.4 Discussion

We previously found that TL was longer at baseline in cases with IMDs, but that this difference did not remain 12 months later (Kalungi *et al*., 2019). The present study built on these results and investigated whether selected polymorphisms within *TERT* and *TERC* interacted with IMDs to influence TL, where *TERT* rs2736100 and *TERC* rs16847897 significantly moderated the association between IMDs and TL at 12 months. To our knowledge, this is the first sub-Saharan African study to investigate these interactions among HIV+ children and adolescents.

Telomeres are maintained by the telomerase enzyme, an enzyme whose catalytic protein component and RNA template is encoded by *TERT* and *TERC*, respectively (Blackburn, Greider, & Szostak, 2006; Wang & Meier., 2004). Given the role that TERT and TERC play in the structure of telomerase (Chen & Greider, 2003; Lee & Blackburn, 1993), variations in genes encoding these components could influence TL. However, none of the investigated polymorphisms significantly influenced the association between IMDs and TL at baseline. We found a significant interaction between IMDs and *TERT* rs2736100 and *TERC* rs16847897 on TL at 12 months. This interaction points to a possible direct influence of IMDs on TL shortening. It is possible that chronic pathophysiological processes characteristic of IMDs, including inflammation, oxidative stress and HPA axis dysregulation (Yu & Woo, 2016), produced a cumulative burden over the one-year study period, with the resultant increase in allostatic load driving TL attrition. Plots of the interaction between IMDs and TL reveal that

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there is no difference in 12 months TL between cases and controls for participants carrying *TERT* rs2736100 *GT or TT* genotype. Similarly, for *TERC* rs16847897, the *GC* or *GG* genotypes appear to be associated with reduced TL at 12 months. Although we found no significant interaction between IMDs and the selected SNPs on TL change, *TERT* rs2736100 and *TERC* rs16847897 interacted with IMDs to influence 12 months TL, results of which depict these genotypes as accelerators of TL attrition among cases of IMDs. However, it is hard to tease out the true effects of the genotypes on TL attrition in the present study, since the reduction in TL after 12 months was strongly significant in both cases and controls ($p < 0.001$ respectively), possibly due to the short one year study period that is not long enough to clearly show the difference in TL attrition between cases and controls. Future studies with a longer study period are required to confirm our findings.

Although the functionality of *TERT* rs2736100 and *TERC* rs16847897 SNPs is not well known, data from previous studies strongly suggest their involvement in disease-associated TL attrition (sporadic idiopathic pulmonary fibrosis), as well as pathophysiological mechanisms, such as inflammation, that are relevant to IMDs (Ding *et al*., 2013; Mushiroda *et al*., 2008; Armanios *et al*., 2007). The rs2736100 SNP is located within intron 2 of *TERT*, a position that has been described to be a putative regulatory region (Taylor *et al*., 2006). The SNP has been reported as a critical factor in TERT synthesis and activation (Melin, Nordfjäll, Andersson & Roos, 2012) and has previously been associated with diseases characterized by TL attrition, including lung cancer (McKay *et al*., 2008) and sporadic idiopathic pulmonary fibrosis (Armanios *et al*., 2007; Mushiroda *et al*., 2008; Tsakiri *et al*., 2007). TERT has also been reported to interact with natural factor kappa B (NF-κb) p65, where it activates NF-κb and increase metalloproteinases in cancer cells (Ding *et al*., 2013). On the other hand, *TERC* rs16847897 has also been reported as locus that probably regulates TL. The *TERC* rs16847897 *CC* genotype has been associated with both shorter TL and lower TERT levels (Al Khaldi, Mojiminiyi, AlMulla, & Abdella, 2015) while each copy of *TERC* rs16847897 major *G*-allele (*CG* or *GG*) was also associated with shorter mean TL in a Han Chinese population (Shen *et al*., 2011).

TERT rs2736100 and *TERC* rs18647897 appear to have regulatory functions or may be in LD with variants that have regulatory functions and thus require further study. Although the nature of the interaction between IMDs and *TERT* and *TERC* on TL is not known, IMDs have been associated with increased oxidative stress (Ng, Berk, Dean & Bush, 2008; Sarandol *et al*., 2007) and inflammatory markers, such as C-reactive protein the pro-inflammatory cytokines

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interleukin-6 and tumor necrosis factor alpha (Musinguzi *et al*, 2018; Miller, Maletic & Raison, 2009). Oxidative stress leads to TL shortening through the inhibition of telomerase activity (Yu & Woo, 2016; Kurz *et al*., 2004; von Zglinicki, 2002). We hypothesize that IMD-related increases in oxidative stress and inflammatory responses produce a cumulative pathophysiological burden. The effect of this on TL may potentially be influenced by genetic variation in *TERT* and *TERC*.

As per the interaction plots, we observed an association between *TERC* rs16847897 *C*-allele and longer TL among the study participants (cases and controls), an observation that is contrary to a large genome-wide meta-analysis that reported an association between *TERC* rs16847897 *G*-allele with longer TL (Codd *et al*., 2013). We have no direct explanation for this observation. However it is important to note that the association of the *C*-allele with longer TL is an outcome of a longitudinal interaction of the SNP with IMDs. The nature of this interaction needs to be elucidated in order to draw conclusions. It is also noteworthy that HIV infection has been associated with TL shortening (Alejos *et al*., 2019; Shiau *et al*., 2018; Auld *et al*., 2016) and thus HIV infection and its associated co-infections may have strongly confounded our findings. Future studies that control for these studies or studies among general populations are required for better comparison of findings.

Our study presents with limitations which deserve mention, and results should be interpreted keeping these in mind. We defined our cases as those individuals diagnosed with depressive disorder, any anxiety disorder or PTSD. The inclusion of PTSD (n = 60) in this sample is contentious, as the disorder has recently been excluded from the IMD categorization in the DSM-5 (American Psychiatric Association, 2013). This may have affected our findings. In order to elucidate the independent contribution of each particular disorder, future studies should analyze each IMD separately. Also, the qPCR efficiencies were outside the optimal range of 90 – 110% (please see addendum E for the standard curves and the dissociation graphs) (Čepin, 2017). The possible reasons for this observation have been discussed under section 5.7 of this PhD thesis. In addition, we did not control for population stratification at analysis since the study participants belonged to the Bagandan population group for which principal components analysis on GWAS data of a sample of over 4,000 individuals has shown that they are genetically similar, as principal components 1 and 2 have been reported to have explained 0.3% and 0.1% of the genetic variation in a Bagandan general population cohort (Gurdasani *et al*., 2019). However, there is a possibility that some participants may not have been Baganda

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although they identified as Baganda and their inclusion could have caused population admixture that could have led to spurious results. Future studies should endeavor to control for population stratification.

Despite the limitations, our study has strengths that are worth mentioning. First, the study sample size was large enough (368 cases and 368 controls) to allow a sufficient power of greater than 80% for the association between IMDs and TL. Second, we measure TL at baseline and 12 months, a longitudinal aspect that allowed us to assess for causation.

3.5 Conclusions

We observed *TERT* rs2736100 and *TERC* rs16847897 produce effects on 12-month TL in Ugandan HIV+ children and adolescents diagnosed with IMDs. Functional studies on telomerase, *TERT* and *TERC* are needed to provide insights on the mechanisms underlying the SNP x IMD interactions on TL in our study group. Understanding how IMDs interact with genetic polymorphisms to influence TL may explain the accelerated aging due to IMDs and may elucidate the nature of comorbidity observed between IMDs and age-related diseases such as diabetes and cardiovascular disease.

3.6 Methods

3.6.1 Study design

This case-control study was carried out in children (aged 5-11 years) and adolescents (12-17 years). A total of 368 cases with any IMD and 368 age- and sex-matched controls were included. Both cases and controls were Ugandans. This study was nested within the previously described CHAKA study (Kinyanda *et al*., 2019; Mpango *et al*., 2017), which enrolled 1339 HIV+ children and adolescents (855 children and 484 adolescents) in Uganda.

3.6.2 Study population

Study subjects were recruited from two HIV clinics in urban Kampala (Joint Clinic Research Centre (JCRC) and Nsambya Home Care) and three HIV clinics in rural Masaka (The AIDS Support Organization (TASO), Kitovu Mobile Clinic and Uganda Cares). All study subjects were on anti-retroviral therapy.

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3.6.3 Procedures

As part of CHAKA study, children and assenting adolescents, as well as their caregivers, were interviewed using a structured questionnaire and provided a blood specimen (4 ml) for genetic analyses. The questionnaire included, amongst others, socio-demographic characteristics and depression, PTSD and anxiety modules of the DSM-5 referenced Child and Adolescent Symptoms Inventory-5 (CASI-5) (Gadow & Sprafkin, 2013). The CASI-5 was administered by trained psychiatric nurses and psychiatric clinical officers at two time points (baseline and 12 months). The CASI-5 lists the symptoms of a wide range of psychiatric disorders including MDD, generalized anxiety disorder, PTSD and attention-deficit/hyperactivity disorder among others. Individual CASI-5 items are rated on a four-point frequency of occurrence scale ranging from never (0) to very often (3). Though there are several CASI-5 scoring algorithms, in the present study we used symptom count cut-off scores, which reflect the prerequisite number of symptoms for a clinical diagnosis. At each study visit, 4 ml of blood from each study participant was collected via venipuncture into an EDTA vacutainer and subsequently stored at −80°C pending DNA extraction.

3.6.4 Inclusion and exclusion criteria

Inclusion criteria: i) HIV-infected outpatients, registered with the HIV Clinics at JCRC or Nsambya Home Care at the Kampala study site and TASO, Kitovu mobile or Uganda Cares clinic at the Masaka site; ii) aged between 5 and 17 years at the time of enrolment; iii) conversant in English or Luganda, the language into which the assessment tools were translated; and iv) able to provide written informed consent/assent. Cases were HIV+ children and adolescents who had any depressive disorder (depression or dysthymia [persistent depressive disorder]), anxiety disorder or PTSD. Controls were age- and sex- matched HIV+ children and adolescents without any psychiatric disorder. Persistent IMDs were baseline cases that remained cases at 12 months while remitted ones were baseline cases
that no longer qualified for a diagnosis at 12 months. *Exclusion criteria*: i) seriously ill and unable to understand study procedures; and ii) any other psychiatric disorder other than the IMDs listed above.

3.6.5 Ethical considerations

The study complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The CHAKA study obtained ethical and scientific clearance from the Uganda Virus Research Institute (UVRI) Science and Ethical Committee (#GC/127/15/06/459) and the

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Uganda National Council of Science and Technology (# HS 1601). The present study obtained approval from the Higher Degrees Research & Ethics Committee, School of Biomedical Sciences, College of Health Sciences, Makerere University (# SBS 421) and the Health Research Ethics Committee of Stellenbosch University (#S17/09/179). Study subjects who were diagnosed with significant psychiatric problems were referred to mental health units at Entebbe and Masaka government hospitals.

3.6.6 Selection of cases and controls

All baseline cases of IMDs (n = 368) in the parent study, CHAKA were considered and thus cases were HIV+ children and adolescents who had any Internalizing mental disorder (IMD). All cases at baseline were ascertained, and the cases were then stratified by site (one of two sites), sex, age category (one of three categories) and Socio-economic status (SES) (one of three SES categories). This resulted in a total of 36 strata (2X2X3X3). In each stratum the number of cases was ascertained (e.g. for males in site 1 in the youngest age category and the lowest SES group there were 9 cases). An equal number of controls (HIV+ children and adolescents without any psychiatric disorder) were then randomly sampled from the stratum concerned (so for males in site 1 in the youngest age category and the lowest SES group we sampled 9 controls), thus the controls were frequency matched to the cases on site, sex, age and SES.

3.6.7 Determination of genotypes for selected polymorphisms in telomerase reverse transcriptase gene and telomerase RNA component gene and analysis for linkage disequilibrium

DNA samples were genotyped for each selected SNP in each of *TERT* (rs2736100, rs7726159, rs10069690 and rs2853669) and *TERC* (rs12696304, rs16847897 and rs10936599), using a kompetitive allele-specific PCR (KASP) assay (LGC, Middelsex, United Kingdom). This genotyping chemistry allowed for bi-allelic discrimination of SNPs although none of the SNPs was validated. The genotyped SNPs in each of *TERT* and *TERC* were analyzed for LD using Haploview software, version 4.2 (Barrett, 2009).'

3.6.8 Power of the study

We calculated the *post hoc* power for our study based on results from a study by Epel *et al.* (2004). We used the formula of sample size and power for difference in means in case–control studies. We worked on the assumption that cases (individuals with IMDs) would have higher

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levels of stress than controls (individuals without IMDs). Epel *et al*. (2004) found a 15% reduction in mean TL among cases compared with controls. Given a 1:1 ratio of cases to controls and using a 5% level of significance, with 368 cases and 368 controls, our study was well powered (power greater than 80%) to detect any reduction above 4.75% in mean TL between cases and controls. For instance, a reduction of 5% in mean TL between cases and controls provided a power of 83.8%.

3.6.9 Statistical methods

Statistical analyses were conducted using Stata 15 (StataCorp, TX, USA). Socio-demographic characteristics (including socio-economic status) were described between cases and controls. Socio-economic status (SES) was generated from a scale of 9 household items owned (car, motorcycle, refrigerator, electricity, bicycle, radio, telephone, cupboard and flask). Each item was weighted in the respective order, a car carrying a maximum weight of 9 and a flask a minimum weight of 1. A total score of items was generated, whose median cut-off of 13 was used to classify low and high SES. A score less than 13 was classified as low SES, while that greater than 13 was classified as high SES. Our study group (Kinyanda *et al*., 2011a) has previously used household items as a measure of SES in rural settings of Uganda. A t-test was used to compare CD4 counts between cases and controls to account for any disparity in HIV disease progression.

Associations between the different socio-demographic factors and TL were tested using one-way analysis of variance (ANOVA) to determine potential confounders. Independent sample t-tests were used to assess the association between IMDs and TL both at baseline and 12 months. *TERT* and *TERC* genotypes were assessed for HWE using a likelihood ratio test. The genotypes were not validated as genotyping was done by a service provider using an automated SNP genotyping array. A two-way ANOVA was used to assess for interaction between each of the polymorphisms and IMDs on TL both at baseline and 12 months, with Bonferroni corrections for multiple testing. To perform a Bonferroni correction, the p-value threshold of 0.05 was divided by 2 (number of separate tests) to yield a corrected threshold p-value of 0.025. These interactions were performed on all the explanatory variables even without observing significant main effects in order to rule out the possibility of cross over interaction where significant interactions may be observed for non-significant main effects. For significant interactions, mean TL at 12 months was plotted against genotypes to elucidate the nature of the interaction terms. The TL change variable was constructed by subtracting the baseline TL value

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from each 12 month TL value for each study participant. Where required, 95% confidence intervals were calculated.

3.7 Abbreviations

µL: microliter; CASI-5: Child and Adolescent Symptom Inventory – edition 5; CD4: cluster of differentiation 4; Ct: threshold cycle; DNA: deoxyribonucleic acid; DSM-V: Diagnostic and statistical manual of mental disorders – edition V; *HBG*: human β-globin gene; HIV+ children and adolescents: HIV+ children and adolescents; HIV/AIDS: human immunodeficiency virus/Acquired immunodeficiency disease syndrome; HIV+: human immunodeficiency virus – positive; IMDs: Internalizing mental disorders; JCRC: Joint Clinical Research Centre; MDD: major depressive disorder; MRC/DfID: Medical Research Council/Department for International Development; ng: nano gram; qPCR: quantitative polymerase chain reaction; TL: relative telomere length; s: seconds; S: single copy gene; SNP: single nucleotide polymorphism; TASO: The AIDS Support Organization; TL: telomere length; TPH2: tryptophan hydroxylase 2; UVRI: Uganda Virus Research Institute

3.8 Acknowledgements

Study subjects, Research assistants of the mental health project of MRC/UVRI & LSHTM Uganda Research Unit, Joint Clinical Research Centre, Nsambya Home Care, TASO – Masaka, Kitovu Mobile Clinic, Uganda Cares – Masaka, Members of the Neuropsychiatric Genetics Laboratory at Stellenbosch University., Data and Statistics Section of the MRC/UVRI & LSHTM Uganda Research Unit, the National Research Foundation of South Africa.

3.9 Funding

The study was funded by Medical Research Council / Department for International Development – African Leadership Award to Prof. Eugene Kinyanda, grant number: MR/L004623/1, the Alliance for Global Health and Science of the Center for Emerging and Neglected Diseases, grant number: 50288/N7145, the South African Research Chairs Initiative in Post-traumatic Stress Disorder, funded by the Department of Science and Technology and the National Research Foundation of South Africa and the Africa Center of Excellence in Materials, Product Development and Nanotechnology (MAPRONANO ACE). AK received a doctoral bursary from the National Research Foundation of South Africa and is supported by both the South African Research Chairs Initiative in Post-traumatic Stress Disorder and the mental health project of MRC/UVRI and LSHTM Uganda Research unit. The funders played

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no role in the design of the study, collection, analysis and interpretation of the data or writing of the manuscript.

3.10 Availability of data and materials

All information gathered about study subjects and their samples is confidential, with access limited to the research team. However, upon request, data from the MRC/UVRI and LSHTM Uganda Research Unit is currently accessed under a data sharing policy via: http://www.mrcuganda.org/sites/default/files/publications/MRC_UVRI_Data_sharing_policy_December2015.pdf.

3.11 Consent for publication

No details, images or videos relating to any of the study subjects are included in this manuscript.

3.12 Ethics approval and consent to participate

The study obtained ethics approval from the Health Research Committee of Stellenbosch University (# S17/09/179) and the higher Degrees Research & Ethics Committee, School of Biomedical Sciences, College of Health Sciences, Makerere University (# SBS 421). The parent study (CHAKA) obtained ethics approval from the Uganda Virus Research Institute (UVRI) Science and Ethical Committee (# GC/127/15/06/459) and the Uganda National Council of Science and Technology (# HS 1601). All caregivers provided written informed consent for their children or adolescents to participate in the study and for a blood specimen to be withdrawn from them for the TL and other genetics analyses. Adolescents further provided written informed assent to participate in the study.

3.13 Author's contribution

Concept: AK, SMJH, EK, SS; Data collection: AK, EK, SMJH, JSW, SS, Data analysis: WS, AK, RNN, SMJH, JSW, SS, MK, JL, EK; First draft: AK, SMJH, JSW, WS, EK, SS, MLJ; Final revision: AK, SMJH, JSW, EK, SS, WS, MLJ, RNN, PK, MK, JL; All authors read and approved the final manuscript.

3.14 Competing interests

The authors declare that they have no competing interests.

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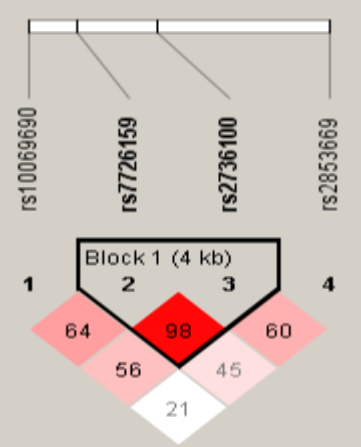
3.15 Supplementary materials

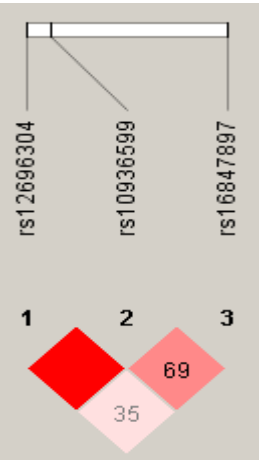
Figure S3.1

LD plot showing linkage disequilibrium (LD) for *TERT* rs10069690, rs7726159, rs2736100 and rs2853669.



The linkage disequilibrium map of *TERT*. D' values are depicted in the diamonds with darker colours depicting stronger LD. The LD map was created using the default Gabriel LD (Gabriel *et al*., 2002), implemented in Haploview, version 4.2 (Barrett, 2009). *TERT* rs7726159 and rs2736100 were in LD resulting in *CT*, *CG* and *AG*. Location = 5' to 3' on chromosome 5.

Figure S3.2



The linkage disequilibrium map of *TERC*. D' values are depicted in the diamonds with darker colours depicting stronger LD. The LD map was created using the default Gabriel LD (Gabriel *et al*., 2002), implemented in Haploview, version 4.2 (Barrett, 2009). None of the SNPs were in LD. Location = 5' to 3' strand on chromosome 3.

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Table S3.1: Two-way analysis of variance for the interaction of IMDs with selected SNPs in telomerase reverse transcriptase gene and telomerase RNA component genes on baseline telomere length, 12 months telomere length and telomere length change

		Baseline telomere length				12 months telomere length				Telomere length change		
SNP	**Obs**	**Variable**	**F**	**P>F**	**Obs**	**Variable**	**F**	**P>F**	**Obs**	**Variable**	**F**	**P>F**
TERT rs2853669	585	IMDs	4.64	0.032	495	IMDs	0.02	0.887	444	IMDs	2.47	0.117
		rs2853669	0.86	0.424		rs2853669	0.75	0.475		rs2853669	0.68	0.506
		IMDs*rs2853669	1.10	0.333		IMDs*rs2853669	0.26	0.770		IMDs*rs2853669	1.22	0.297
TERT rs7726159	606	IMDs	10.8	0.001	526	IMDs	4.32	0.038	459	IMDs	0.26	0.609
		rs7726159	0.21	0.809		rs7726159	0.01	0.986		rs7726159	0.19	0.830
		IMDs*rs7726159	1.16	0.316		IMDs*rs7726159	1.32	0.267		IMDs*rs7726159	0.01	0.986
TERT rs10069690	600	IMDs	12.03	<0.001	513	IMDs	1.85	0.175	454	IMDs	0.76	0.384
		rs10069690	0.32	0.729		rs10069690	0.27	0.760		rs10069690	0.44	0.644
		IMDs*rs10069690	0.53	0.589		IMDs*rs10069690	1.96	0.142		IMDs*rs10069690	1.57	0.210
TERC rs12696304	597	IMDs	8.63	0.003	512	IMDs	2.43	0.120	451	IMDs	0.18	0.668
		rs12696304	0.23	0.979		rs12696304	0.02	0.978		rs12696304	0.03	0.972
		IMDs*rs12696304	0.33	0.720		IMDs*rs12696304	1.79	0.167		IMDs*rs12696304	1.37	0.255
TERC rs10936599	597	IMDs	2.90	0.089	516	IMDs	0.72	0.397	454	IMDs	0.00	0.958
		rs10936599	1.01	0.314		rs10936599	2.40	0.122		rs10936599	0.45	0.503
		IMDs*rs10936599	0.01	0.921		IMDs*rs10936599	0.02	0.878		IMDs*rs10936599	0.09	0.767

SNP = single nucleotide polymorphism, Obs = number of observations.

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CHAPTER FOUR

This chapter highlights the role of chronic stress and genetic variants in *SLC6A4* and *TPH2* on the association between acute stress and IMDs among the study participants. It is based on a manuscript that is scheduled for submission to Frontiers in Genetics (Impact factor, 3.517).

The *5-HTTLPR*-rs25531 *S-A-S-A* haplotype and chronic stress moderate the association between acute stress and internalizing mental disorders among HIV+ children and adolescents in Uganda

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Key words: Internalizing mental disorders, acute stress, serotonin transporter gene, *5-HTTLPR*-rs25531, chronic stress, HIV+ children and adolescents, Uganda

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4.1 Abstract

Background

Internalizing mental disorders (IMDs) among HIV+ children and adolescents are associated with poor disease outcomes, such as faster HIV disease progression. Although it has been suggested that the development of IMDs is moderated by interaction of stressful life events and vulnerability (genetic and or acquired) factors, which are, in turn, modulated by resilience factors, the underlying etiology, including biochemical or molecular events, are largely unknown. This study investigated the association between acute stress and IMDs, and moderation by chronic stress and genetic variants in the serotonin transporter (*SLC6A4*) and tryptophan hydroxylase 2 (*TPH2*) genes.

Hypothesis

We hypothesized that acute stress acts through genetic and environmental vulnerability factors to increase the risk of developing IMDs.

Methods

Polymorphisms in *SLC6A4* (*5-HTTLPR*, rs25531, *5-HTTLPR*-rs25531 and *STin2* VNTR) and *TPH2* (rs1843809, rs1386494, rs4570625 and rs34517220) were genotyped in 368 HIV+ children and adolescents (aged 5-17 years) with any IMD (depression, anxiety disorders or post-traumatic stress disorder), and 368 age- and sex-matched controls. Chronic and acute stress categories were derived by hierarchical cluster analysis, using a composite index that assessed social disadvantage variables. Logistic regression analysis was used to assess the independent moderating effect of chronic stress and each selected polymorphism on the association between acute stress and IMDs.

Results

We observed a statistically significant association between severe acute stress and IMDs ($p = 0.001$). The likelihood of having an IMD increased with increasing acute stress, where HIV+ children and adolescents who experienced severe acute stress were twice as likely to develop IMDs compared to HIV+ children and adolescents who experienced mild acute stress ($p = 0.001$). Chronic stress was found to interact with severe acute stress to increase the risk of IMDs ($p = 0.033$). Acute stress was found to interact with *5-HTTLPR*-rs25531 *S-A-S-A* haplotype to increase the risk for IMDs among Uganda HIV+ children and adolescents (p =

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0.049). We found no evidence for a combined interaction of acute stress, chronic stress and *5-HTTLPR*-rs25531 on IMDs.

Conclusions

The odds of having an IMD were higher among HIV+ children and adolescents who experienced severe acute stress compared to HIV+ children and adolescents who experienced mild acute stress. Chronic stress and *5-HTTLPR*-rs25531 independently moderated the association between acute stress and IMDs.

4.2 Background

HIV-positive (HIV+) children and adolescents suffer a considerable burden of internalizing mental disorders (IMDs) (Kinyanda *et al*., 2019; Mellins *et al*., 2012; Nachman *et al*., 2012). IMDs are characterized by quiet, internal distress (Tandon, Si, & Luby, 2011) and include depressive and anxiety disorders, as well as posttraumatic stress disorder (PTSD) (American Psychiatric Association, 2013). IMDs among people living with HIV/AIDS have generally been associated with a number of negative outcomes, including more rapid HIV disease progression (Chida & Vedhara, 2009; Ironson *et al*., 2005), poor adherence to medication (Kinyanda *et al*., 2018; Springer, Dushaj & Azar, 2012), risky sexual behavior (Kinyanda *et al*., 2018; Springer, Dushaj & Azar, 2012) and poor linkages to care for newly diagnosed persons with HIV (Bhatia *et al*., 2011).

IMDs are complex disorders with gene-environment interactions contributing to their etiology (Musci, Augustinavicius & Volk, 2019). The behavioral disturbances characteristics of these disorders are likely due to a large number of genes, with each having only a small effect size on phenotype (Assary, Vincent, Keers & Pluess, 2018; Plomin & Davis, 2009). The role of psychosocial factors and their interaction with biological mechanisms in the etiology of IMDs is still poorly understood. HIV+ children and adolescents experience various chronic life stressors such as awareness of their HIV-status, increased levels of stigma and poorer parental mental health (Betancourt *et al*., 2014). As chronic stressors are reported to be risk factors for IMDs (Revenson *et al*., 2016; Adelman *et al*., 2014; Robles, Slatcher, Trombello, & McGinn, 2014), chronic stressors likely contribute to the etiology of IMDs among HIV+ children and adolescents (Lwidiko *et al*., 2018; Boyes & Cluver, 2015).

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IMDs are highly heritable (Smoller *et al*., 2016): twin studies have estimated a genetic heritability of 35% for depression (Otte *et al*., 2016) and 30-50% for post-traumatic stress disorder (PTSD) (Smoller *et al*., 2016). A meta-analysis of family and twin studies estimated a genetic heritability of 31.6% for generalized anxiety disorder (GAD) (Hettema, Neale & Kendler, 2001). A more recent GWAS of monozygotic and dizygotic female twins has estimated a genetic heritability of 42% for GAD (Davies *et al*., 2015). Despite being highly heritable, the underlying etiology of IMDs is largely unknown although dysregulation in serotonergic transmission has been implicated in IMDs like depression among adults living with HIV (Hammoud *et al*., 2010).

The serotonergic system in the central nervous system is extremely important in regulating mood and anxiety (Olivier *et al*., 2015). After an impulse is fired and serotonin (5-HT) is released into the synaptic cleft, 5-HT re-uptake reduces the activity of the serotonergic neurons, preparing the neuron for a new discharge (Olivier *et al*., 2015; Artigas, 2013a, b). Encoded by the serotonin transporter gene (solute carrier family 6 member 4, *SLC6A4)*, the serotonin transporter (5-HTT) influences serotonergic transmission by regulating the duration of serotonin in the synaptic cleft (Kristensen *et al*., 2011; Rudnick, 2006). 5-HTT is a target for selective serotonin re-uptake inhibitors (SSRIs) that competitively block substrate binding and thereby prolong neurotransmitter action at the synapse (Cipriani *et al*., 2018; Kristensen *et al*., 2011). Tryptophan hydroxylase 2 (TPH2), encoded by *TPH2*, catalyzes the rate-limiting step in 5-HT biosynthesis (Carkaci-Salli *et al*., 2006; Walther *et al*., 2003) and is expressed exclusively in the brainstem (Kennedy *et al*., 2012), an area which is the major locus of serotonin-producing neurons (Kennedy *et al*., 2012). Long-term treatment with the SSRI fluoxetine has been found to be associated with concurrent upregulation of *TPH2* messenger ribonucleic acid (RNA) expression and alleviation of depressive symptoms (Shishkina *et al*., 2007), indicating that low *TPH2* messenger RNA expression could represent a dysregulation corrected by fluoxetine. The 5-HTT and TPH2 are thus important proteins in the availability and transmission of 5-HT in the brain.

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SLC6A4 is located on chromosome 17 (US National Library of Medicine, 2019). Within the promoter region of the *SLC6A4* is the serotonin transporter-linked polymorphic region (*5-HTTLPR*), which has been implicated in depression (Wang *et al*., 2016; Clarke *et al*., 2010), anxiety-related traits (Munafò *et al*., 2009b) and PTSD (Xie *et al.* 2009). *5-HTTLPR* variants comprise either 14 (short, *S*-allele) or 16 (long, *L*-allele) copies of a 22-23 base pair (bp) imperfect repeat (Heils *et al*., 1996). *In vitro* studies show that the *L*-allele has two-to-three times higher basal transcriptional activity compared to the *S*-allele (Lesch *et al*., 1996; Philibert *et al*., 2008). In proximity to *5-HTTLPR* is an *A* to *G* single nucleotide polymorphism (SNP), which has been reported to alter expression of the *SLC6A4* by creating a functional AP2 transcription-factor binding site (Ehli *et al*., 2012; Hu *et al*., 2006). The *G*-allele has been associated with reduced reporter gene expression. This SNP, when analyzed in combination with *5-HTTLPR*, results in *L-A* and *L-G* haplotypes. The *L-G* haplotype has been shown to have lower expression levels compared to the *L-A* haplotype *(*Hu *et al*., 2006; Lipsky, Hu & Goldman, 2009). However, some studies found no effect of the *L-G* haplotype on *SLC6A4* expression (Philibert *et al*., 2008; Martin *et al*., 2007).

Also contained within *SLC6A4* is a variable number of tandem repeats (VNTR) polymorphism. Located in the second intron (*STin2*), it consists of multiple repeated copies of a 16-17 bp element (Furlong *et al*., 1998; Battersby *et al*., 1996). Three alleles have been reported (National Center for Biotechnology Information, 2019), containing 9 (*STin2.9*), 10 (*STin2.10*) and 12 (*STin2.12*) copies of the repeat. The alleles have been associated with differential expression, with *STin2.9* associated with increased *SLC6A4* expression, with increasing number of repeats associated with reduced reporter gene expression in rat neonate prefrontal cortical cultures (Ali *et al*., 2010). The *STin2* VNTR polymorphism has also been found to interact with the *5-HTTLPR* polymorphism to regulate expression of *SLC6A4* with the combination of the *5-HTTLPR S*-allele and either *STin2.10* or *STin2.12* associated with increased expression (Ali *et al*., 2010; Haddley *et al*., 2011). The *STin2.9* allele has been found to be associated with anxiety in patients with self-harming behaviors (Evans, Li, & Whipple, 2013), *STin2.10* has been found to associated with both anxiety and PTSD (Xiao *et al*., 2019; Evans, Li & Whipple, 2013) and the *STin2.12* allele has been found to be associated with depression, neuroticism and suicide (Kalungi *et al*., 2017 de Lara *et al*., 2007; O'Gara *et al*., 2008).

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The tryptophan hydroxylase 2 gene is located on chromosome 12 at position 12q21.1. Intronic *TPH2* rs1843809 and rs1386494, and the intergenic rs4570625 SNP have been found to be associated with depression (Gao *et al*., 2012; Zill *et al*., 2004; Anttila *et al*., 2009). The rs34517220 SNP has been reported to modulate *TPH2* expression through altering the binding sites for foxa1 and foxa2 transcription factors (Pristerà *et al*., 2015). Foxa2 plays a role in establishing progenitor domains for serotonergic neuron precursors in the ventral hindbrain and in activating transcription factors required for the terminal differentiation of serotonergic neurons (Jacob *et al*., 2007). Therefore, rs34517220-driven variation in Foxa2 binding could have important effects on the development of neural serotonergic systems.

The diathesis-stress hypothesis of neuropsychiatric disorders postulates that a lower stress threshold is required for psychiatric disease to occur in individuals who harbor certain vulnerability factors, which may be genetic and/or acquired (Caspi *et al*., 2003; Silberg *et al*., 2001; Monroe & Simons, 1991) (Figure 4.1). Stress represents a prevalent environmental risk factor for a number of mental disorders, including depression, anxiety and PTSD (Popoli *et al*., 2012; de Kloet *et al*., 2005) and it is currently accepted that gene-environment interactions underlie the etiology of many, if not all, of the IMDs (Jawahar, Toben, & Baune, 2019; Willis & Brock, 2019; Caspi & Moffitt, 2006).

In the context of the diathesis-stress hypothesis, with acute stress representing the exposure variable, we investigated polymorphisms in *SLC6A4* (*5-HTTLPR*, rs25531, and *STin2* VNTR) and *TPH2* (rs1843809, rs1386494, rs4570625 and rs34517220) as the genetic vulnerability factors and chronic stress as the acquired vulnerability factor for IMDs in a sample of HIV+ children and adolescents. Our *a priori* selection of these polymorphisms was based on their association with IMDs (*5-HTTLPR*, *STin2* VNTR, *TPH2* rs1843809, rs1386494, rs4570625 and rs34517220) and regulation of *SLC6A4* (*STin2* VNTR, rs25531).

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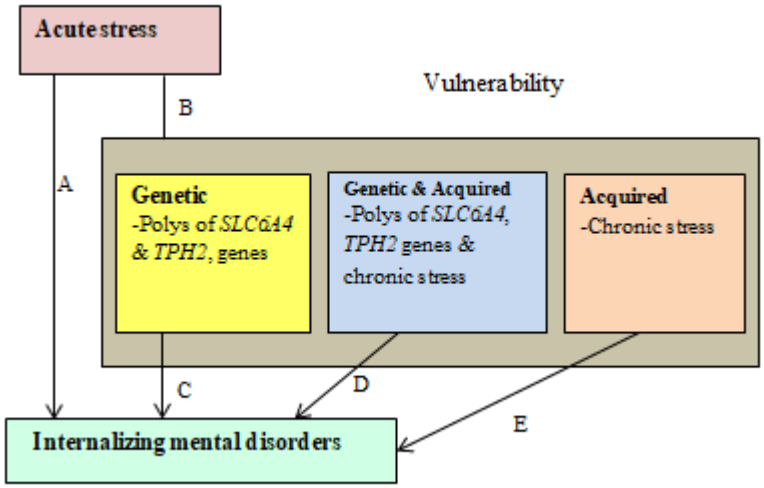

Figure 4.1: The conceptual framework for the present investigation, based on the diathesis-stress model.

The conceptual framework is adapted from the diathesis-stress model for depression (Monroe & Simons, 1991). The aim of the present study was to investigate the association between acute stress and IMDs (A). We postulated that acute stress would interact with vulnerability factors (B) (genetic (yellow block), acquired (pink block) or both (blue block)) to increase the risk for IMDs.

4.3 Methods

4.3.1 Study design

This was a case-control study nested within a Medical Research Council/Department for International Development (MRC/DfID)-funded project that investigated mental health among HIV+ children and adolescents in Kampala and Masaka districts of Uganda (CHAKA study). The present study selected from the 1339 Ugandan HIV+ children and adolescents (aged 5 – 17 years) of black African ancestry enrolled in the CHAKA study (Kinyanda *et al*., 2019). Of the 1339, all participants with any internalizing mental disorder (IMD) (368 cases) who met inclusion criteria (see below) and an equal number of age-, site-, socio-economic status (SES)- and sex- matched controls were selected. Cases were defined as subjects who had any depressive disorder (depression or dysthymia [persistent depressive disorder]) or any anxiety disorder (generalized anxiety disorder, separation anxiety disorder, social anxiety disorder, panic disorder and agoraphobia) or PTSD. Controls were age-, site-, SES- and sex-matched participants, without any psychiatric disorder. Genomic DNA was extracted from an archived cell pellet sample for each participant.

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4.3.2 Study population

Study participants for the CHAKA study were recruited from two HIV clinics in urban Kampala (Joint Clinic Research Centre and Nsambya Home Care) and three HIV clinics in rural Masaka (The AIDS Support Organization, Kitovu mobile clinic and Uganda Cares clinic). Study staff visited the patient waiting areas of each clinic, introduced the study and requested participants enroll in the study. At each of the study sites, HIV+ children and adolescents/caregiver dyads who agreed to participate in the study were then screened for suitability according to the eligibility criteria (Kinyanda *et al*., 2019). All study participants were on antiretroviral therapy. The study participants were of Bagandan ethnicity, the largest ethnic group in Uganda. The study participants were thus expected to be genetically similar since relatively modest genetic differentiation has been observed among populations representing the major sub-populations in sub-Saharan Africa (Gurdasani *et al*., 2015).

4.3.3 Procedures

Children and assented adolescents, as well as their caregivers, were interviewed using a structured questionnaire. The questionnaire included, among others, socio-demographic characteristics (sex, study site, age, caregiver level of education and SES) and modules on depression, anxiety and PTSD from the DSM-5 referenced Children and Adolescent Symptom Inventory 5 (CASI-5; caregiver reported) (Gadow & Sprafkin, 2013) and the Youth Inventory-4R (YI-4R; youth reported) (Gadow & Sprafkin, 1999).

Both the CASI-5 and the YI-4R were locally adapted for use in Uganda by the CHAKA study team (Mpango *et al*., 2017). The assessment tools were administered by trained psychiatric nurses and psychiatric clinical officers at two time points (baseline and 12 months). The CASI-5 and YI-4R list the symptoms of a wide range of psychiatric disorders including major depressive disorder, generalized anxiety disorder, PTSD and attention-deficit/hyperactivity disorder, among others. Individual CASI-5 items are rated on a 4-point frequency of occurrence scale ranging from never (0) to very often (3). Though there are several CASI-5 scoring algorithms, in the present study we used symptom count cut-off scores that reflect the prerequisite number of symptoms for a clinical diagnosis.

At each study visit, 4 ml of blood was withdrawn from each study participant by venipuncture into an EDTA vacutainer and was stored at −80 °C pending DNA extraction.

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4.3.4 Inclusion and exclusion criteria

The following were the inclusion and exclusion criteria for the CHAKA study:

Inclusion criteria: i) HIV-infected outpatients, registered with an HIV clinic at any of the study sites; ii) aged between 5 and 17 years at the time of enrolment; iii) conversant in English or Luganda, the language into which research assessment tools were translated; and iv) able to provide written informed consent (caregiver) and assent (adolescents). Exclusion criteria: i) seriously ill ii) being unable to understand study procedures.

4.3.5 Ethical considerations

Both CHAKA and the present study were conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The CHAKA study obtained ethical and scientific clearance from the Uganda Virus Research Institute's Science and Ethical Committee (# GC/127/15/06/459) and the Uganda National Council of Science and Technology (# HS 1601). The present study obtained approval from the Higher Degrees Research & Ethics Committee of the School of Biomedical Sciences, Makerere University (# SBS 421) and the Health Research Ethics Committee of Stellenbosch University (# S17/09/179). All caregivers provided informed consent for their children / adolescents to participate in the study and for a blood specimen to be drawn from them (child/adolescent) for genetics analyses. Adolescents further provided informed assent to participate in the study. Study participants who were diagnosed with significant psychiatric problems were referred to mental health units at Entebbe and Masaka government hospitals.

4.3.6 Selection of cases and controls

All baseline cases of IMDs (n = 368) in the CHAKA study were considered and thus cases were HIV+ children and adolescents who had any IMD. All cases at baseline were ascertained, and the cases were then stratified by site (one of two sites), sex, age category (one of three categories) and SES (one of three SES categories). This resulted in a total of 36 strata (2 x 2 x 3 x 3). In each stratum the number of cases was ascertained (e.g. for males in site 1 in the youngest age category and the lowest SES group there were 9 cases). An equal number of controls (HIV+ children and adolescents without any psychiatric disorder) were then randomly sampled from the stratum concerned (so for males in site 1 in the youngest age category and the lowest SES group we sampled 9 controls), thus the controls were frequency matched to the cases on site, sex, age and SES.

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4.3.7 DNA extraction

Archived whole blood samples were retrieved and DNA was extracted from each retrieved sample using the QiAmp Mini DNA Extraction Kit according to manufacturer's instructions (Qiagen GmbH, Germany).

4.3.8 Determining *SLC6A4* genotypes

All polymerase chain reactions (PCR) for *SLC6A4* polymorphisms were performed in a GeneAmp PCR System 9700 (Perkin Elmer Biosystems, Foster City, CA, USA). PCR was carried out in 25 µl reaction volumes containing: DNA template, 200 µM dNTP (Kapa Biosystems, Cape Town, South Africa), 5 µl of 10X Taq DNA polymerase buffer (Kapa Biosystems), 1.0 mM magnesium chloride (Kapa Biosystems), 0.625 units (U) Taq DNA polymerase (Kapa Biosystems), and 0.5 µM of each primer (Integrated DNA Technologies, Coralville, Iowa, United States), with bi-distilled water.

The *5-HTTLPR*, *5-HTTLPR*-rs25531 and *STin2* VNTR polymorphisms were genotyped following a procedure described by Kalungi *et al*. (2017). Fragment sizes revealed by PCR were confirmed by restriction fragment length analysis on the ABI prism. Expected fragment sizes of the alleles at the *5-HTTLPR*-rs25531 locus were as follows: *S-A* = 281 bp, *L-A* = 325 bp and *S-G-L-G* = 151 bp, resulting into the following genotypes: *S-A-S-A* = 281 bp; *L-A-L-A* = 325 bp; *S-G-S-G*, *L-G-L-G*, *L-G-S-G* = 151 bp; *L-G-S-A* = 151 bp + 281 bp and *L-A-S-G*, *L-A-L-G* = 325 bp + 151 bp. Expected fragment sizes of the alleles at the STin2 VNTR locus were as follows: 9-repeat (*STin2.9*) = 250 bp, 10-repeat (*STin2.10*) = 265 bp and 12-repeat (*STin2.12*) = 300 bp, resulting in the following genotypes: 9/9 = 250 bp, 9/10 = 250bp + 265bp, 9/12 = 250 bp + 300 bp, 10/10 = 265 bp, 10/12 = 265 bp + 300bp, and 12/12 = 300 bp.

4.3.9 Determining genotypes for selected SNPs in tryptophan hydroxylase 2 gene

DNA samples were quantified and sent to LGC laboratory (LGC, Middlesex, United Kingdom) for automated SNP genotyping using the kompetitive allele-specific PCR (KASP) assay. Genotypes were determined for *TPH2* rs1843809, rs1386494, rs4570625 and rs34517220. However none of these genotypes were validated.

Because the Bantu group of people in East Africa have been reported to genetically cluster together as per principal components analysis (Unpublished results on NeuroGAP data, provided by Anne Stevenson & Elizabeth Atkinson), a minor allele frequency cut-off of at least

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0.1 was set for each selected SNP based on the Luhya, a population that belongs to same Bantu group as the population of the present study. The minor allele frequency data were obtained from an online genomic project (Clarke *et al*., 2017).

4.3.10 Haplotype analyses

We used Haploview version 4.2 (Barrett, 2009) to analyze for linkage disequillibrium (LD). *SLC6A4* analyses included 5-*HTTLPR*, rs25531 and *STin2* VNTR polymorphisms. As Haploview relies on allelic discrimination, the *5-HTTLPR* and *STin2* VNTR were coded as dummy variables i.e. *S* = 1, *L* = 2 and *STin2.10* = 1 and *STin2.12* = 2. As very few participants carry the *STin2.9* allele (n = 6), these participants were coded as missing *STin2* VNTR data. *TPH2* analyses included the three alleles genotyped. Participants with more than 50% missing genotypes were excluded from the analysis, yielding data from 692 participants for each of the *SLC6A4* and *TPH2* analyses. None of the investigated *TPH2* polymorphisms were in LD while *SLC6A4 5-HTTLPR* and rs25531 were in LD (Supplementary materials, Figures S4.1 and S4.2).

4.3.11 Generation of acute and chronic stress class categories

Social disadvantage variables were grouped into an index of acute and chronic stress. Caregiver mental state (assessed as psychological distress using the Self-report Questionnaire-20 (Beusenberg, Orley & World Health Organization, 1994)), child–caregiver relationship (assessed as child-caregiver interactions, using data on how often the caregiver i) beats, ii) insults, iii) spanks or iv) yells at the child/adolescent) and HIV symptoms were grouped together to constitute ‘acute stress’ and orphanhood, study site (urban vs rural) and caregiver level of education as variables constituting ‘chronic stress’. Variables were scored on a disadvantage scale where, for example, double orphanhood carried a higher chronic stress score vs. single orphanhood or not orphaned; food availability: not enough food carried a higher chronic stress score vs. enough food; study site: urban carried a higher chronic stress score than rural; and caregiver level of education: no formal education carried a higher chronic stress scores than primary and primary a higher stress score than secondary etc. Hierarchical cluster analysis using Statistica 13.5 software (TIBCO, CA, USA), Euclidian distance, as distance measure and Ward’s method for clustering (Ward, 1963), was used to generate the different cut-off points for each acute and chronic stress class respectively.

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The acute stress index ranged from 0 to 2.46, with a normal distribution, while the chronic stress index ranged from 0 to 3.75, with a normal distribution as well. A total of 3 classes were generated for each type of stress by the hierarchical cluster analysis i.e. mild, moderate and severe. For acute stress, the mild class had an acute stress score of less than 0.362, the moderate class a score of 0.362 to 0.622, while the severe class a score of greater than 0.622. For chronic stress, the mild class had a chronic stress score of less than 1.375, the moderate class a score of 1.375 to 2.375, while the severe class a score of greater than 2.375.

4.3.12 Power of the study

Using Stata 15 (StataCorp, TX, USA) software, we did a *post hoc* power calculation. Given a case-control ratio of 1:1, and assuming a zero correlation of exposure between cases and controls, a probability of 0.5 of exposure among controls at a 0.05 significance level, and an expected odds ratio at least 1.8 for IMDs under severe acute stress, we needed 188 cases to achieve a power of 80% (Lachin, 1992). Our sample of 368 cases was therefore adequately powered.

4.3.13 Statistical methods

Statistical analyses were conducted using Stata 15 (StataCorp, TX, USA). Socio-demographic characteristics were compared between cases and controls. SES was generated from a scale of 9 household items owned (car, motorcycle, refrigerator, electricity, bicycle, radio, telephone, cupboard and flask). Each item was weighted in the respective order, a car carrying a maximum weight of 9 and a flask a minimum weight of 1. A total score of items was generated; with a median value of 13. A score less than 13 was classified as low SES while that greater than 13 was classified as high SES. A T-test was used to compare the distribution of cluster of differentiation 4 (CD4) counts between cases and controls. We computed 95% confidence intervals and statistical significance was set at a p-value less than or equal to 0.05.

Genotype distributions were compared between cases and controls. Likelihood-ratio tests were used to test genotypes for the Hardy-Weinberg equilibrium (HWE) in both cases and controls. Logistic regression was used to assess the relationship between *TPH2* rs1843809 and IMDs (Equation for the model was $Y = \beta_0 + \beta_1 X_1$, where, Y = IMDs, β_0 = constant, X_1 = rs1843809, and $\beta 1$ = coefficient for rs1843809). Logistic regression models were used to assess the relationship between acute stress and IMDs (Equation for the model was $Y = \beta_0 + \beta_1 X_1$, where, Y = IMDs, β_0 = constant, X_1 = acute stress, and $\beta 1$ = coefficient for acute stress).

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The moderating effect of chronic stress on the association between acute stress and IMDs was assessed by comparing logistic regression models of the association between acute stress and IMDs with and without chronic stress. Interactions between acute stress and chronic stress were tested using a likelihood-ratio test (equation for the model was $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_1X_2$, where Y = IMDs, β_0 = constant, X_1 = acute stress, X_2 = chronic stress and β_1 to β_3 are the coefficients for acute stress, chronic stress and the interaction term respectively).

The moderating effect of each genotype for each of the selected polymorphisms on the association between acute stress and IMDs was also assessed by comparing logistic regression models of the association between acute stress and IMDs with and without the polymorphism controlling for chronic stress. Interactions between acute stress and each of the polymorphism were tested using a likelihood-ratio test, controlling for chronic stress (equation for the model was $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2$, where, Y = IMDs, β_0 = constant, X_1 = acute stress, X_2 = polymorphism/SNP, X_3 = chronic stress and β_1-β_4 are the coefficients for acute stress, the polymorphism/SNP, chronic stress and the interaction respectively). These interaction models were performed on all the explanatory variables even without observing significant main effects in order to rule out the possibility of cross over interaction where significant interactions may be observed for non-significant main effects. Three-way interactions of acute stress, chronic stress and any selected polymorphism on IMDs were not assessed as the two-way models were better than the three-way models. The 95% confidence intervals were calculated for all analyses.

4.4 Results

Socio-demographic factors were similarly distributed between cases and controls as shown in Table 4.1.

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Table 4.1: Distribution of socio-demographic factors in cases and controls

Variable (n)	Case n (%)	Control n (%)	p-value
Sex			0.111
Male (342)	160 (43.6)	182 (49.5)	
Female (393)	207 (56.4)	186 (50.5)	
Site			0.941
Urban (415)	208 (56.5)	207 (56.3)	
Rural (321)	160 (43.5)	161 (43.7)	
Age			0.374
7-11 years (389)	202 (57.6)	187 (54.2)	
12-17 years (307)	149 (42.4)	158 (45.8)	
Education level			0.371
No formal education (13)	9 (2.5)	4 (1.1)	
Primary (648)	323 (88.0)	325 (88.8)	
Secondary (72)	35 (9.5)	37 (10.1)	
Socioeconomic status			0.459
Low (332)	171 (46.5)	161 (43.8)	
High (404)	197 (53.5)	207 (56.2)	
Mean CD4 count at baseline	947.04	944.02	0.939

CD4, cluster of differentiation 4; primary, 0 – 7 years of formal education; Secondary, 8 – 14 years of formal education; Low socioeconomic status, 0 – 13; High socioeconomic status, > 13. All numbers that do not add up to the total of 736 participants were due to missing data.

The HWE p-values among cases and controls for each polymorphism are shown in Table 4.2. None of the genotype distributions deviated significantly from HWE among controls except for rs1386494, which was thus removed from further analysis. For investigated *SLC6A4* polymorphisms, *5-HTTLPR* was in LD with rs25531 (see Supplementary Material Figure S4.1), while for *TPH2* SNPs, none of the polymorphisms were in LD (see Supplementary Material Figure S4.2). The association between each investigated polymorphism and IMDs is shown in Table 4.2. Participants with *TPH2* rs1843809 *TG* and *TT* genotypes were protective against IMDs as compared with participants with the *GG* genotype (Table 4.3).

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Table 4.2: Distribution and Hardy-Weinberg analysis of genotypes between cases and controls for selected polymorphisms in *SLC6A4* and *TPH2*

Genotype	Cases, n (%)	Controls, n (%)	P-value*	HWE Cases	HWE Controls
5-HTTLPR (n =698)			0.286	0.323	0.1916
LL	223 (62.3)	231 (67.9)			
LS	115 (32.1)	94 (27.7)			
SS	20 (5.6)	15 (4.4)			
rs25531 (n =685)			0.988	0.795	0.641
AA	227 (65.4)	221 (65.4)			
AG	108 (31.1)	106 (31.4)			
GG	12 (3.5)	11 (3.2)			
5-HTTLPR-rs25531 haplotype (n = 685)			0.688	N/A	N/A
L-A-L-A	130 (37.4)	136 (40.2)			
L-A-L-G	76 (21.9)	82 (24.3)			
L-A-S-A	79 (22.8)	71 (21.0)			
L-A-S-G	2 (0.6)	Absent			
L-G-L-G	12 (3.5)	11 (3.3)			
S-A-S-A	18 (5.2)	15 (4.4)			
S-A-L-G	30 (8.6)	23 (6.8)			
STin2 VNTR (n = 687)			0.203	0.090	0.440
10/10	26 (7.5)	28 (8.8)			
10/12	126 (36.3)	112 (35.3)			
12/12	189 (54.5)	177 (55.9)			
9/9	5 (1.4)	Absent			
9/12	1 (0.3)	Absent			
rs1843809 (n = 686)			0.003	0.009	0.537
TT	105 (30.1)	113 (33.5)			
TG	150 (43.0)	169 (50.2)			
GG	94 (26.9)	55 (16.3)			
rs1386494 (n = 685)			0.06	0.956	0.007
GG	210 (59.7)	202 (60.7)			
GA	124 (35.2)	125 (37.5)			
AA	18 (5.1)	6 (1.8)			
rs34517220 (n = 685)			0.945	0.557	0.913
AA	90 (25.6)	84 (25.1)			

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AG	170 (48.4)	166 (49.7)			
GG	91 (25.9)	84 (25.2)			
rs4570625 (n = 683)			0.574	0.787	0.229
GG	88 (25.2)	90 (26.9)			
GT	177 (50.7)	156 (46.7)			
TT	84 (24.1)	88 (26.4)			

5-HTTLPR = serotonin transporter linked polymorphic region, *STin2* VNTR = Serotonin transporter intron 2 variable number of tandem repeats, HWE = Hardy-Weinberg equilibrium, N/A = not applicable. P-value* = p-value for association of each investigated polymorphism with IMDs.

Table 4.3: Association between tryptophan hydroxylase 2 gene rs1843809 and internalizing mental disorders

IMDs	Odds ratio	P>|Z|	95% confidence interval
rs1843809 *GG*	Reference		
rs1843809 *TG*	0.52	0.001	0.349 – 0.774
rs1843809 *TT*	0.54	0.005	0.355 – 0.832

IMDs = internalizing mental disorders.

Table 4.4 determines the association between acute stress and IMDs while table 4.5 determines the independent effect of chronic stress and SLC6A4 polymorphisms on the association seen in Table 4.4 (between acute stress and IMDs). A significant association was observed between acute stress and IMDs (Table 4.4).

Table 4.4: Association between acute stress and IMDs

Acute stress class	OR (IMDs)	P > |Z|	95% CI	P-value
Mild	Reference			
Moderate	1.1	0.687	0.758 – 1.523	0.001
Severe	1.9	0.001	1.298 – 2.651	

OR = odds ratio; IMDs = internalizing mental disorders, CI = confidence interval

Apart from the *5-HTTLPR*-rs25531 haplotype (Table 4.5), we found no significant interactions between acute stress and any of the investigated polymorphisms on IMDs (supplementary materials, Table S4.1). Significant interactions between acute and chronic stress (p = 0.033) and acute stress and *5-HTTLPR*-rs25531 *S-A-S-A* haplotype (p = 0.049) were observed on

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IMDs (Table 4.5). The odds of an IMD were 4.3 times higher among participants under both severe acute stress and severe chronic stress as compared to those under mild acute stress and mild chronic stress (Table 4.5). The odds for being a case of an IMD were 14.8 times higher in participants with the *S-A-S-A* haplotype with moderate acute stress compared to participants with the *L-A-L-A* haplotype and mild acute stress. Similarly, the odds of having an IMD were 11.95 times higher in *S-A-S-A* participants with severe acute stress compared to *L-A-L-A* participants with mild stress (Table 4.5).

Table 4.5: Logistic regression analyses for the interaction of acute stress with *5-HTTLPR*-rs25531 and chronic stress respectively on IMDs

Model	Variable	Odds ratio	P>|Z|	95% CI	P-value
Excluding any polymorphism or chronic stress	Mild acute stress	Reference			0.046[a]
	Moderate acute stress	1.22	0.282	0.848 - 1.761	
	Severe acute stress	1.92	0.001	1.327 - 2.785	
Including 5-*HTTLPR*-rs25531	**Acute stress*5-*HTTLPR*-rs25531**				
	Mild AS**L-A-S-G*	1			0.049[b]
	Moderate AS**L-A-L-G*	2.52	0.022	1.142 – 5.540	
	Moderate AS**L-A-S-A*	1.60	0.235	0.736 – 3.497	
	Moderate AS**L-A-S-G*	0.63	0.124	0.351 – 1.135	
	Moderate AS**L-G-L-G*	0.83	0.870	0.092 – 7.535	
	Moderate AS**S-A-L-G*	1.48	0.563	0.395 – 5.500	
	Moderate AS**S-A-S-A*	14.83	0.030	1.294 -170.010	
	Severe AS**L-A-L-G*	3.72	0.002	1.630 – 8.474	
	Severe AS**L-A-S-A*	2.82	0.013	1.248 – 6.350	
	Severe AS**L-A-S-G*	1.16	0.629	0.641 – 2.091	
	Severe AS**L-G-L-G*	0.85	0.872	0.121 – 6.010	
	Severe AS**S-A-L-G*	1.58	0.493	0.426 – 5.881	
	Severe AS**S-A-S-A*	11.95	0.011	1.755 –81.331	
Including chronic stress	**Acute stress*Chronic stress**				
	Moderate AS*moderate CS	1.04	0.896	0.607- 1.768	0.033[c]
	Moderate AS*severe CS	1.80	0.035	1.041 – 3.106	
	Severe AS*moderate CS	0.95	0.904	0.406 – 2.217	
	Severe AS*severe CS	4.28	0.001	1.832 – 10.012	

AS = acute stress, CS = chronic stress, Acute stress**5-HTTLPR*-rs25531 = interaction between acute stress and *5-HTTLPR*-rs25531 genotypes on IMDs, [a] = the model without chronic stress/*SLC6A4*, [b] = p-value for the likelihood-ratio test of interaction between acute stress and

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5-HTTLPR-rs25531 on IMDs, [c] = p-value for the likelihood-ratio test of interaction between acute stress and chronic stress on IMDs.

4.5 Discussion

This study investigated the association between acute stress and IMDs, and whether this association is moderated by chronic stress, or genetic variants in *SLC6A4* and *TPH2*. To our knowledge, this is the first sub-Saharan African study to investigate these interactions among HIV+ children and adolescents. Results revealed a statistically significant association between acute stress and IMDs. This association was found to be moderated by *5-HTTLPR*-rs25531 in a haplotype-dependent manner. Specifically, in comparison to *L-A-L-A* haplotype carriers with experience of mild acute stress, individuals carrying the *S-A-S-A* haplotypes were more likely to have an IMD under conditions of moderate and severe acute stress. We found no evidence for a significant combined interaction of acute stress, chronic stress and *5-HTTLPR*-rs25531 on IMDs.

Stress represents a prevalent environmental risk factor for many mental disorders, including depression and anxiety (Popoli *et al*., 2012; de Kloet *et al*., 2005). Acute stress has been reported to be associated with the likelihood of developing IMDs among samples of adolescent school children and exposed adult disaster workers (Brown *et al*., 2016; O'Connor, Rasmussen, & Hawton, 2010; Fullerton, Ursano & Wang, 2004). In line with previous studies, where acute stress has been associated with IMDs such as depression and PTSD (Fullerton, Ursano & Wang, 2004), and anxiety (Grillon *et al*., 2007), we found that risk for IMDs increased with increasing acute stress and was highest for severe acute stress. Chronic stress was found to significantly moderate the association between acute stress and IMDs. Previous studies have reported on chronic stress as a risk factor for IMDs (Adelman *et al*., 2014; Evans, Li, & Whipple, 2013; Revenson *et al*., 2016; Robles, Slatcher, Trombello, & McGinn, 2014; de Kloet *et al*., 2005; Charney & Manji, 2004). Vulnerability to IMDs by chronic stress (through interaction with acute stress) could be due to alterations in the functioning of the HPA that follow exposure to chronic stress (de Kloet *et al*., 2005; Fuchs & Flïugge, 2006). In addition, chronic stress has been reported to affect serotonergic signaling in the brain (van den Buuse & Hale, 2019). As serotonergic systems have been implicated in threat appraisal, psychophysiological measures of stress response (skin conductance and startle reactions), and attentional bias to negative stimuli, it is possible that alterations in 5-HT signaling due to chronic stress may at least partially contribute to some of the behavioral features of IMDs

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(Klumpers *et al*., 2015; Klumpp *et al*., 2014; Pergamin-Hight, Bakermans-Kranenburg, van IJzendoorn, & Bar-Haim, 2012).

In contrast to previous findings of associations between *5-HTTLPR* and *STin2* VNTR and IMDs (Xiao *et al*., 2019; Wang *et al*., 2016; Smoller *et al*., 2016; Gressier *et al*., 2013; Evans, Li & Whipple, 2013; Zhao *et al*., 2012; Battersby *et al*., 1996), we found no direct association between any of the investigated *SLC6A4* polymorphisms and IMDs. This may be due to the complex genetic etiology of IMDs, where the combination of individual genetic variants each of small effect size ultimately contributes to the risk of developing an IMD (Plomin & Davis, 2009) and thus the effect of one gene may not be strong enough to show significant association. Indeed, studies examining the contributions of *5-HTTLPR* and *STin2* variants have yielded inconsistent results with other previously published work similarly failing to find a significant association (Xiao *et al*., 2019; Culverhouse *et al*., 2018; Munafò *et al*., 2009a; Munafò *et al*, 2009b). In order to resolve these contradictory results, studies looking at the effect of more than one gene (GWAS) are required.

Based on the diathesis-stress hypothesis of neuropsychiatric disorders, we postulated that the association between acute stress and IMDs would be moderated by genetic polymorphisms, chronic stress or a combination of genetic polymorphisms and chronic stress. Results revealed chronic stress and the *5-HTTLPR*-rs25531 *S-A-S-A* haplotype to each significantly moderate the association between acute stress and IMDs.

The interaction between the *5-HTTLPR* and stress has previously been investigated in gene-environment studies of IMDs (Sharpley *et al.*, 2014; Karg, Burmeister, Shedden & Sen, 2011). In contrast to previous studies that have reported the moderating role of *5-HTTLPR* on the association between stress and IMDs (Conway, Slavich & Hammen, 2014; Sharpley *et al*., 2014; Karg, Burmeister, Shedden & Sen, 2011; Caspi *et al*., 2003), we found no significant moderating effect of *5-HTTLPR* on the association between acute stress and IMDs. However, our results revealed that the combined *5-HTTLPR*-rs25531 haplotype significantly moderates the association between acute stress and IMDs. These results revealed the *S-A-S-A* genotype as a vulnerability factor for IMDs. HIV+ children who experienced moderate and severe acute stress and possessed the *S-A-S-A* haplotype were more likely to have an IMD, compared to HIV+ children who experienced mild acute stress and possessed the *L-A-L-A* haplotype.

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Previous studies have reported the *SS* genotype to be a risk for different IMD psychopathologies including depression (Cervilla *et al*., 2007; Zalsman *et al*., 2006; Haberstick *et al*., 2016), anxiety (Armbruster *et al*., 2009) and IMDs in general (Conway, Slavich & Hammen, 2014). Contrary to meta-analyses that found the *S*-allele to confer risk for depression in subjects experiencing stressful life events (Sharpley *et al*., 2014; Karg, Burmeister, Shedden & Sen, 2011), we found no association between the *SS* genotype and IMDs in the present study and this observation could be due to low power to detect the effect of *5-HTTLPR* on the association between acute stress and IMDs among our study participants. Our study sample size archived a *post hoc* power of greater than 80% under assumption that there was zero correlation of exposure between cases and controls and that the probability of exposure among controls was 0.5. There is however a possibility that the probability of exposure could have been less than 0.5 among controls, thus reducing the power for the present study. The association between *SS* genotype and IMDs has been suggested to be due to its lower transcriptional activity (Seripa *et al*., 2013; Iurescia *et al*., 2012; Hu *et al*., 2006; Baca-García *et al*., 2002). Future functional studies that examine the functional effects of the *S-A-S-A* compared to the *L-A-L-A* haplotype would be required for us to determine whether lower 5-HTT levels are driving our results.

A significant association was observed between *TPH2* rs1843809 and IMDs. The functional role of *TPH2* rs1843809 is currently not known. Being an intronic variant, it is less likely that rs1843809 would have marked effect on *TPH2* expression or TPH2 structure, as introns are spliced during mRNA processing, although potential effects of intronic variants have been suggested (Kleinjan & van Heyningen, 2005). It is also possible that rs1843809 is in linkage disequilibrium with a causal variant. For example, rs1843809 is in LD with a missense rs142055199 SNP among the Luhya, a population that speaks the same Niger-Congo language as the population of the present study (Baganda) (Countries & their cultures, 2019). East African populations that speak this class of language have been found to be genetically similar by principal components analysis on GWAS data from NeuroGAP pilot study (Unpublished results provided by Anne Stevenson & Elizabeth Atkinson). The rs142055199 is located in the zinc finger C3H1-type containing gene, which modulates interleukin-8 (IL-8) transcription (National Center for Biotechnology Information, 2019). Modulation of IL-8 would be of interest since IMDs have been associated with inflammatory processes (Wang *et al*., 2019; Hori & Kim, 2019; Musinguzi *et al*., 2018; Miller, Maletic & Raison, 2009). Further studies will be needed to determine the mechanisms underlying the influence of this SNP in IMDs.

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None of the investigated polymorphisms in *TPH2* moderated the association between acute stress and IMDs. This suggests that although TPH2 is critical in 5-HT biosynthesis (Carkaci-Salli *et al*., 2006; Walther *et al*., 2003), genetic variants may either not necessarily moderate an individual's response to stress or that the investigated polymorphisms may not be the causal polymorphisms that moderate an individual's response to stress.

4.6 Limitations and recommendations

Although we found the association between acute stress and IMDs to be moderated via vulnerability as we had hypothesized, our study presents with the following limitations. We defined IMDs as having any depressive disorder, anxiety disorder or PTSD. As much as anxiety and PTSD are commonly comorbid with depression, the inclusion of PTSD (n = 60) is contentious as it has been excluded from the IMD category in the Diagnostic and Statistical Manual of Mental Disorders – 5th edition (DSM-5) (American Psychiatric Association, 2013). Although the inclusion of PTSD samples allowed the present study to achieve the desired statistical power, a low correlation between genetic risk factors for PTSD and those of depression and anxiety would reduce the power to detect true significant associations and interactions. Future studies should endeavor to stratify analyses according to IMD although this will require larger sample sizes. In addition, both acute and chronic stress indices were measured using a number of context-specific indicators. This approach was adopted because there is no locally adapted tool for assessing such in this setting and the variables used to generate these indices are known stressors in this population. However, this tool remains to be validated. Moreover, we did not control for population stratification at analysis. The study participants belong to the Bagandan population group, for which principal components analysis on a GWAS sample of over 4,700 individuals has shown that they are genetically similar (unpublished data). There is, however, a possibility that some participants were not Bagandan even though they could speak Luganda (the main language of Baganda), indicating that we may have had an admixed sampled. Future studies should therefore control for population stratification, in order to circumvent confounding of results due to possible ancestry associated differences in genetic architecture. Also, due to the exploratory nature of the study, multiple testing corrections were not done. Correcting for multiple testing would have rendered the interaction analysis non-significant. We therefore acknowledge the dangers of false positive findings in this study which are potentially important clinical findings need to be verified by follow-up research.

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4.7 Conclusions

Acute stress was found to be associated with an increased risk of IMDs, and this association was found to be moderated by the 5-*HTTLPR*-rs25531 *S-A-S-A* haplotype and chronic stress among Ugandan HIV+ children and adolescents. These results support the diathesis-stress model, though the mechanisms through which acute stress interacts with *5-HTTLPR*-rs25531 *S-A-S-A* haplotype to moderate the risk of IMDs need to be elucidated. This will allow for interventions to be targeted to at-risk individuals, an important consideration in resource constrained settings.

4.8 Acknowledgements

The assistance of the following are gratefully acknowledged: study subjects, research assistants of the mental health section of MRC/UVRI & LSHTM Uganda Research Unit, Joint Clinical Research Centre, Nsambya Home Care, TASO – Masaka, Kitovu Mobile Clinic, Uganda Cares – Masaka, members of the Neuropsychiatric Genetics Laboratory at Stellenbosch University, Data and Statistics Section of the MRC/UVRI & LSHTM Uganda Research Unit; and the National Research Foundation of South Africa.

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4.10 Availability of data and materials

All information gathered about study subjects and their samples is confidential, with access limited to the research team. However, upon request, data from the MRC/UVRI and LSHTM Uganda Research Unit is currently accessed under a data sharing policy via:

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http://www.mrcuganda.org/sites/default/files/publications/MRC_UVRI_Data_sharing_policy_December2015.pdf.

4.11 Consent for publication

No details, images or videos relating to any of the study subjects are included in this manuscript.

4.12 Ethics approval and consent to participate

The study obtained ethics approval from the Health Research Committee of Stellenbosch University (# S17/09/179) and the higher Degrees Research & Ethics Committee, School of Biomedical Sciences, College of Health Sciences, Makerere University (# SBS 421). The parent study (CHAKA) obtained ethics approval from the Uganda Virus Research Institute (UVRI) Science and Ethical Committee (# GC/127/15/06/459) and the Uganda National Council of Science and Technology (# HS 1601). All study subjects provided informed consent/assent to participate in the study and for a blood specimen to be withdrawn from them for the genetics analyses.

4.13 Author's contribution

Concept: AK, SMJH, EK, SS; Data collection: AK, EK, SMJH, SS, JSW; Data analysis: WS, AK, RNN, SMJH, JSW, SS, MK, EK; First draft: AK, SMJH, SS, EK, JSW, MLJ, WS, RNN, MK, PK; Final revision: AK, SMJH, JSW, EK, SS, WS, MLJ, RNN, PK, MK; all authors read and approved the final manuscript.

4.14 Competing interests

The authors declare that they have no competing interests.

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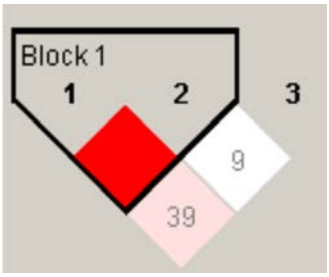
4.15 Supplementary materials

Supplementary Material

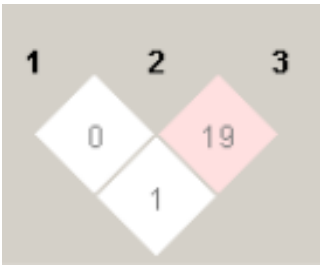
Figure S4.1

LD plot showing linkage disequilibrium (LD) for *SLC6A4* 5-*HTTLPR*, rs25531 and *STin2* VNTR



Linkage disequilibrium map of *SLC6A4*. D' values are depicted in the diamonds with darker colors depicting stronger LD. The LD map was created using the default Gabriel LD (Gabriel *et al*., 2002), implemented in Haploview, version 4.2 (Barrett, 2009). 1 = rs25531, 2 = *5-HTTLPR*, 3 = *STin2* VNTR. *5-HTTLPR* and rs25531 were in LD. Location = 5' to 3' on chromosome 17.

Figure S4.2



Linkage disequilibrium map of *TPH2*. D' values are depicted in the diamonds with darker colors depicting stronger LD. The LD map was created using the default Gabriel LD (Gabriel *et al*., 2002), implemented in Haploview, version 4.2 (Barrett, 2009). 1 = rs4570625, 2 = rs1843809 and 3 = rs34517220. None of the SNPs were in LD. Location = 5' to 3' strand on chromosome 12.

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Table S4.1: Logistic regression analysis for the interaction of acute stress on selected serotonin transporter gene and tryptophan hydroxylase 2 gene polymorphisms on IMDs

Model	Variable	Odds ratio	P>|Z|	95% CI	P-value
Excluding any polymorphism	Mild acute stress	Reference			0.046[f]
	Moderate acute stress	1.22	0.282	0.848 - 1.761	
	Severe acute stress	1.92	0.001	1.327 – 2.785	
Including rs1386494	**Acute stress*rs1386494**				
	Moderate AS**GA*	1.17	0.889	0.125 – 11.004	0.786[f]
	Moderate AS**GG*	1.88	0.574	0.208 – 17.067	
	Severe AS**GA*	0.73	0.808	0.056 – 9.508	
	Severe AS**GG*	0.94	0.963	0.074 – 11.990	
Including rs1843809	**Acute stress*rs1843809**				
	Moderate AS**TG*	1.93	0.174	0.750 – 4.951	0.198[f]
	Moderate AS**TT*	3.07	0.031	1.110 – 8.490	
	Severe AS**TG*	0.99	0.988	0.359 – 2.743	
	Severe AS**TT*	1.89	0.253	0.635 – 5.634	
Including rs34517220	**Acute stress*rs34517220**				
	Moderate AS**GA*	1.31	0.544	0.544 – 3.177	0.927[f]
	Moderate AS**GG*	1.48	0.459	0.527 – 4.138	
	Severe AS**GA*	1.37	0.491	0.557 – 3.378	
	Severe AS**GG*	1.23	0.685	0.446 – 3.419	
Including rs4570625	**Acute stress*rs4570625**				
	Moderate AS**TG*	2.26	0.073	0.926 – 5.532	0.390[f]
	Moderate AS**TT*	2.41	0.085	0.886 – 6.568	
	Severe AS**TG*	1.35	0.507	0.554 – 3.299	
	Severe AS**TT*	1.21	0.719	0.423 – 3.480	
Including rs25531	**Acute stress*rs25531**				
	Moderate AS**2*	1.88	0.117	0.853 – 4.153	0.487[f]
	Moderate AS*3	0.77	0.815	0.082 – 7.172	
	Severe AS**2*	1.46	0.362	0.647 – 3.298	
	Severe AS**3*	0.43	0.404	0.058 – 3.418	

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Including *5-HTTLPR*	**Acute stress*5-*HTTLPR***				
	Moderate AS**LS*	1.47	0.346	0.662 – 3.246	0.463[f]
	Moderate AS**SS*	3.08	0.269	0.419 – 22.654	
	Severe AS**LS*	1.31	0.521	0.577 – 2.958	
	Severe AS**SS*	4.15	0.133	0.647 – 26.574	
Including *STin2*.VNTR	**Acute stress*STin2.VNTR**				
	Moderate AS**10/12*	1.79	0.432	0.419 – 7.625	0.203[f]
	Moderate AS**12/12*	1.76	0.429	0.434 – 7140	
	Severe AS**10/12*	0.53	0.429	0.106 – 2.592	
	Severe AS**12/12*	1.14	0.868	0.239 – 5.450	

AS = acute stress, * = interaction, [f] = p-value for the likelihood-ratio test of interaction between acute stress and the selected polymorphism on IMDs.

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CHAPTER FIVE
DISCUSSION

5.1 Conceptualization of the present study

Despite the high prevalence of IMDs and the considerable impact they exert on quality of life, their etiology remains poorly understood. Stressful life events have been reported to play a primary role in the development of depression (Lan, Jia, Lin & Liu, 2019; Cohen *et al*., 2019; Slavich & Irwin, 2014; Hammen & Rudolph, 2003; Kinyanda *et al*., 2017), anxiety (Miloyan *et al*., 2018; Sheerin *et al*., 2018; Abbo *et al*., 2013), and PTSD (Frewen, Zhu & Lanius 2019; Bernstein *et al*., 2005).

The diathesis-stress hypothesis of neuropsychiatric disorders postulates that a lower stress threshold is required for psychiatric disease to occur in individuals who harbor certain vulnerability factors, which may be genetic and/or acquired (Caspi *et al*., 2003; Silberg *et al*., 2001; Monroe & Simons, 1991). IMDs are heritable, with twin studies indicating an estimated genetic heritability of 30–67% (Otte *et al*., 2016; Smoller *et al*., 2016; Domschke & Maron, 2013; Hettema, Neale & Kendler, 2001). However, the genetic risk factors that underlie this heritability are currently not conclusively known. The diathesis-stress hypothesis asserts that acute stress may interact with underlying genetic risk and result in disease (Monroe & Simmons, 1991), hence the importance of studying gene-environment interactions in stress-related disorders like IMDs (Sharma, Powers, Bradley & Ressler, 2016).

In sub-Saharan Africa, research has been undertaken to understand acquired vulnerability to acute stress in the context of IMDs (Kinyanda *et al*., 2017, 2013, 2011a, b; Abbo *et al*., 2013). However, there has been less focus on investigating the genetic vulnerability factors (Popejoy & Fullerton, 2016; Dalvie *et al*., 2015). Investigating genetic and environmental vulnerability (risk) for IMDs amongst African populations is urgently needed: expanding psychiatric genetics studies to include populations of more diverse ancestries will allow a more comprehensive insight into the genetic architecture of IMDs. In particular, African genomes hold a high percentage of private alleles which could be useful in fine-mapping of disease-causing alleles (Campbell & Tishkoff, 2008). To understand the genetic and environmental risk for IMDs in an African population, we elected to study acquired and genetic contributors to IMDs in Ugandan HIV+ children and adolescents.

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5.2 Utility of the measure of acute and chronic stress in the present study

The variables used to create the acute stress index (caregiver mental state, child-caregiver relationship and HIV symptoms) and the chronic stress index (orphanhood, having enough food, study site and caregiver level of education) in the present study have been shown to be associated with IMDs among both children and adolescents in Uganda. For example, food security, study site (rural vs. urban) and adverse life events have been associated with increased risk for depression among HIV+ adults in Uganda (Kinyanda *et al*., 2011b, 2017). Similarly, living without parents, low parental education levels, guardian unemployment and study site were associated with increased risk for both depression and anxiety disorders among children and adolescents in Uganda (Abbo *et al*., 2013; Kinyanda *et al*., 2011a). In addition, lower CD4 nadir was a significant predictor of incident depression among participants of the present study (Kinyanda *et al*. submitted to AIDS and Behavior).

In order to generate the acute stress and chronic stress classes in the present study, hierarchical cluster analysis was employed. This is an algorithm that groups similar objects into clusters whereby each cluster differs from another and the objects within each cluster are nearly similar (Ward, 1963). The acute stress index ranged from 0 to 2.46, with a normal distribution, while the chronic stress index ranged from 0 to 3.75, with a normal distribution as well. A total of 3 classes were generated for each type of stress by the hierarchical cluster analysis i.e. mild, moderate and severe. For acute stress, the mild class had an acute stress score of less than 0.362, the moderate class a score of 0.362 to 0.622, while the severe class a score of greater than 0.622. For chronic stress, the mild class had a chronic stress score of less than 1.375, the moderate class a score of 1.375 to 2.375, while the severe class a score of greater than 2.375.

The derived measure of acute stress associated with IMDs ($p = 0.001$) while the measure of chronic stress showed a trend towards significance ($p = 0.067$). As desired, these two measures were weakly positively correlated with each other ($r = 0.157$). The trend towards significance between the chronic stress index used in this study and IMDs probably reflects a poor fit of the variables used to constitute this index; these variables were selected *a posteriori* from the variables of the main CHAKA study. Despite this shortcoming, this approach could provide a mechanism of assessing stress in African settings, where culturally adapted measures are lacking. There is, however, a need for additional studies to validate these measures.

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5.3 Serotonin and reduced telomere length as likely candidates in internalizing mental disorders

The serotonergic system in the CNS is important in regulating mood and anxiety (Olivier *et al*., 2015) and the 5-HTT is a target for SSRIs (Cipriani *et al*., 2018; Kristensen *et al*., 2011). We therefore elected to examine genetic variants relevant to 5-HT signaling and availability in the brain i.e. *SLC6A4* and *TPH2*, which are responsible for serotonin reuptake and biosynthesis respectively (Carkaci-Salli *et al*., 2006; Walther *et al*., 2003).

We based our additional analyses on TL shortening, which has been associated with IMDs of depression (Verhoeven *et al*., 2017; Lin, Huang & Hung, 2016; Schutte & Maouff, 2015; Garcia-Rizo *et al*., 2013; Verhoeven *et al*., 2014; Shalev *et al*., 2014; Douillard-Guilloux, Guiolloux, Lewis & Sibille, 2013; Kinser & Lyon, 2013), anxiety disorders (Verhoeven *et al*., 2017; Verhoeven *et al*., 2015; Kananen *et al*., 2010) and PTSD (Avetyan, Zakharyan, Petrek & Arakelyan, 2019; Roberts *et al*., 2017; O'Donovan *et al*., 2011; Zhang *et al*., 2014), as well as frequent comorbid age-related diseases (Sanders & Newman, 2013). Compared with the general population, higher mortality rates have been reported among patients with IMDs (Pratt, Druss, Manderscheid & Walker, 2016; Druss *et al*., 2011; Colton & Manderscheid, 2006; Cuijpers & Smit, 2002). This higher mortality has been mainly attributed to age-related diseases, such as cancer, cardiac and cerebrovascular disease (Druss *et al*., 2011; Colton & Manderscheid, 2006; Cuijpers & Smit, 2002) amongst individuals with IMDs, and it has been suggested that IMDs may indeed be risk factors for these age-related diseases (Cohen *et al*., 2015).

Shorter telomeres have been described as a risk factor for many age-related chronic conditions (Sanders & Newman, 2013). The link between IMDs and these age-related diseases has been hypothesized to be mediated via biochemical processes of oxidative stress or inflammation (Yu & Woo *et al*., 2016; Lin *et al*., 2018), and pro-oxidative environments and inflammation have, in turn, been associated with telomere shortening (Wolkowitz *et al*., 2011a; Wolkowitz *et al*., 2011b). IMDs have been associated with inflammatory processes (Wang *et al*., 2019; Hori & Kim, 2019; Musinguzi *et al*., 2018; Miller, Maletic & Raison, 2009) and autonomic nervous system dysfunction (Kop *et al*., 2010). Research on the role of TL in IMDs has attracted attention due to the comorbidity of age-related diseases and IMDs (Cohen *et al*., 2015). The nature of the association between TL and IMDs is unknown. Some studies have hypothesized that shorter TL is a risk factor for IMDs (Gotlib *et al*., 2015; Shalev *et al*., 2014), while others

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have hypothesized that shorter TL and IMDs occur concurrently due to effects of exposure to stress (Needham *et al*., 2015; Zhang *et al*., 2014) or that TL shortening is due an effect of IMDs (Shalev *et al*., 2014; Schutte & Malouff, 2015; Yu & Woo, 2016). As TL attrition may be influenced by these same processes, and is associated with both age-related chronic conditions and IMDs, TL is a valid target for investigation in this study.

The present study investigated the genetic and environmental risk for IMDs among Ugandan HIV+ children and adolescents, and the approach is based on the diathesis-stress model, whereby the risk of developing IMDs is predicated on the presence of vulnerability factors. We hypothesized that acute stress leads to IMDs through vulnerability (both genetic and or acquired). We investigated chronic stress as the acquired (environmental) vulnerability factor, while TL and selected polymorphisms within *SLC6A4* and *TPH2* were investigated as the genetic vulnerability factors. Since the nature of the association between TL and IMDs is largely unknown, we opted to investigate it prior to considering TL as a vulnerability factor. We hypothesized that the association between TL and IMDs (if any) would be moderated by the acquired vulnerability factor of chronic stress and genetic vulnerability by variations in *TERT* and *TERC*.

5.4 Chronic stress moderates the association between acute stress and internalizing mental disorders

In line with previous studies that have reported stress (Kinyanda *et al*., 2011a,b, 2013, 2017; Abbo *et al*., 2013; Popoli *et al*., 2012; de Kloet *et al*., 2005), acute stress disorder (O'Connor, Rasmussen, & Hawton, 2010; Fullerton, Ursano & Wang, 2004) and acute stress (Fullerton, Ursano & Wang, 2004; Grillon *et al*., 2007) as being among the risk factors for IMDs, we found a statistically significant association between acute stress (defined in Section 5.2 above) and IMDs (Chapter 4, Section 4.3). As expected, increasing acute stress increased the risk for IMDs among our study participants. Participants who experienced severe acute stress were twice more likely to be a case of IMDs as compared to participants who experienced mild acute stress (Chapter 4, section 4.3, Table 4.4). We further observed that the association between acute stress and IMDs was significantly moderated by chronic stress. As per the hypothesis that acute stress leads to IMDs through acquired vulnerability by chronic stress, severe chronic stress interacted with severe acute stress to significantly increase the likelihood of any IMD from 1.9 to 4.3. These results are in line with previous studies that have reported chronic stress to be a risk factor for IMDs (de Kloet *et al*., 2005; Charney & Manji, 2004).

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This significant interaction is in agreement with our conceptual framework. HIV+ children and adolescents experience various chronic life stressors, such as awareness of their HIV-status, increased levels of stigma and poor parental mental health (Betancourt *et al*., 2014). These results could potentially explain the increased occurrence of IMDs among HIV+ children and adolescents. Elucidating the mechanisms by which chronic stress confers risk for IMDs could offer insights into biological pathways that lead to the development of these disorders. This is especially important, considering current challenges, including low efficacy and short windows of therapeutic benefit, in treating IMDs (Munkholm, Paludan-Müller & Boesen, 2019; Ollendick & King, 1994). For example, fluoxetine is the only antidepressant that might reduce depressive symptoms among children and adolescents (Ciprian *et al*., 2016), yet studies have reported a lack of difference between fluoxetine and placebo treated groups (Atkinson *et al*., 2014; Emslie *et al*., 2014). Understanding pathways linking stress exposure and IMDs will facilitate the development of new drugs that can better address the symptoms of IMDs.

5.5 Association between internalizing mental disorders and accelerated telomere length attrition: the role of chronic stress and genetic variations in telomerase reverse transcriptase and telomerase RNA component genes

In the present study, mean TL at baseline was significantly different from mean TL at 12 months among a combined sample (cases and controls) of the study participants (Chapter 2, Section 2.4). We investigated whether the difference in mean TL between cases and controls was moderated by genetic variation in genes implicated in TL biology, namely *TERT* and *TERC* (Blackburn, Geider & Szostak, 2006). Contrary to our hypothesis and previous studies that observed shorter TL among cases of IMDs (Avetyan, Zakharyan, Petrek & Arakelyan, 2019; Malouff & Schutte, 2017; Verhoeven *et al*., 2014, 2015, 2017; Lin, Huang & Hung, 2016; Garcia-Rizo *et al*., 2013; Shalev *et al*., 2014; Zhang *et al*., 2014; Douillard-Guiloux *et al*., 2013; Kinser *et al*., 2013; Kananen *et al*., 2010; O'Donovan *et al*., 2011), mean baseline TL was longer in cases compared to controls. We have no direct explanation for this observation although elevated telomerase levels have been reported among people with depression compared to healthy, age- and sex-matched controls (Simon *et al*., 2015; Chen *et al*., 2014; Wolkowitz *et al*., 2012; Damjanovic *et al*., 2007). A potential reason for this could therefore be due to increased levels of telomerase activity in individuals with IMDs, although this remains to be investigated. It has been suggested that increased telomerase levels may represent

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a compensatory effort to prevent the excessive shortening of telomeres that is associated with IMDs (Lin *et al*., 2012; Damjanovic *et al*., 2007). However, it would be prudent to conduct further studies in a similar setting to replicate our results.

On investigating the association between IMDs and TL at 12 months, we found no statistically significant difference in mean TL between cases and controls. Given our results at baseline, we hypothesized that TL length reduced at an increased rate amongst cases compared to controls during the 12 months. It is not known whether the hypothesized accelerated telomere shortening is a direct effect of IMDs, whether the development of IMDs and hypothesized accelerated shortening of telomeres are simultaneous effects of increased stress exposure or whether accelerated telomere shortening is a risk factor for IMDs, as has been hypothesized by previous studies (Gotlib *et al*., 2015; Zhang *et al*., 2014). Our findings potentially suggest a causative role for IMDs in TL shortening, because TL reduced more rapidly in cases compared to controls, supporting the school of thought that accelerated shortening is a direct effect of IMDs (Yu & Woo, 2016; Lindqvist *et al*., 2015).

It has been hypothesized that IMDs increase oxidative stress and chronic inflammation through either dysregulation of the HPA axis or by acting as a psychological stressor that elevates glucocorticoid hormones and lead to reduced antioxidants (Yu & Woo, 2016). Increased oxidative stress and chronic inflammation have been reported to cause TL shortening (Lin *et al*., 2018; Lindqvist *et al*., 2015; Wong *et al*., 2014; Wolkowitz *et al*., 2011a; von Zglinicki, 2002). Despite elevated telomerase levels, faster TL attrition may occur among cases of IMDs. A recent review has hypothesized that accelerated TL attrition that is observed among cases of IMDs despite the elevated telomerase levels, could be brought about by a faster rate of attrition, which supersedes the rate at which telomerase lengthen telomeres (Lindqvist, Simon & Wolkowitz, 2019). Although our findings could be influenced by technical errors during determination of TLs, we had a fairly large sample size (n = 736) that allowed us to achieve a *post hoc* study power of over 83%, using the formula of sample size and power for difference in means in case–control studies, basing on a study by Epel *et al*., 2004. In order to control for intraplate variability, TL for each sample was measured in triplicate and standards were used, the slope of which was used to calculate the concentration vs Ct values. Standards were also repeated across plates in order to control for interplate variability. GenEx software (GenEx User Guide, 2012) was then used to control for both intra- and interplate variability (http://www.gene-quantification.de/datan. html) by analyzing the interplate calibrator,

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correcting for PCR efficiency and normalizing the TL copy number to the *HBG* copy number. It is therefore unlikely that the longer TL observed among cases at baseline or the statistically significant difference in mean baseline and 12 months TL (section 2.4.1, Table 2.3) could have been due to chance, error or laboratory artefact.

Chronic stress has been described as a risk factor for IMDs (Adelman *et al*., 2014; Evans, Li, & Whipple, 2013; Revenson *et al*., 2016; Robles, Slatcher, Trombello, & McGinn, 2014; Charney & Manji, 2004; de Kloet *et al*., 2005) and has been associated with shorter TL (Damjanovic *et al*., 2007; Epel *et al*., 2004; Notterman & Mitchell, 2015; Parks *et al*., 2009; Shalev, 2012; Shalev *et al*., 2013). These associations suggest that the development of IMDs and shortening of TL occur simultaneously following chronic stress exposure. However, the present study provides no evidence for this phenomenon as chronic stress neither associated with TL nor moderated the association between IMDs and TL. The association between IMDs and TL could instead be explained by genetic variation in genes responsible for the formation and function of the telomerase enzyme (discussed in Section 5.5.1 below). Since accelerated TL attrition was an outcome of the presence of IMDs, we hypothesized that TL is not a risk factor for IMDs. Thus we did not include TL in the analyses that investigated the role of vulnerability on the association between acute stress and IMDs.

It should be noted that some other factors may have been responsible for the greater TL reduction among cases than controls over 12 months. For example, participants were all on ART, with the type of ART regimen not accounted for in the analysis. Nucleoside reverse transcriptase inhibitors have been reported to inhibit human telomerase *in vitro* (Hukezalie *et al*., 2012; Leeansyah *et al*., 2013) and may also inhibit telomerase *in vivo*. Furthermore, factors known to affect TL, such as diet (Shiels *et al*., 2011) and frequency of physical exercise (Abrahin, Cortinhas-Alves, Vieira & Guerreiro, 2019; Stenbäck *et al*., 2019; Cherkas *et al*., 2008) were not accounted for due to unavailability of data. We recommend that future studies control for these factors.

Understanding the mechanisms through which IMDs lead to accelerated TL attrition may help to unravel the biochemical processes that take place following onset of IMDs, which may aid the discovery of new drugs or drug targets for better management of these disorders. Furthermore, these mechanisms may also provide insight into the biochemical processes that underlie the comorbidity between IMDs, age-related conditions and metabolic diseases.

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5.5.1 Association between internalizing mental disorders and variants in genes encoding telomerase reverse transcriptase and telomerase RNA component genes

The present study investigated whether genetic or acquired vulnerability (chronic stress) or a combination of both could moderate the association between IMDs and TL observed among our study participants. Significant interactions were observed between two SNPs and IMDs on TL. Specifically, mean TL were not statistically different between cases and controls at 12 months among individuals with *TT* and *TG* genotypes for *TERT* rs2736100 and among individuals with *GG* and *GC* genotypes for *TERC* rs16847897. The significant effect of the interaction between IMDs and genetic variations in *TERT* and *TERC* on TL could potentially explain the lack of association between IMDs and TL at 12 months. We hypothesized that biochemical processes that occur as an effect of IMDs are implicated in TL shortening. IMDs have been reported to lead to impaired allostatic function, resulting in increased insulin resistance, poor mitochondrial health and dysregulation of the autonomic and neuroendocrine stress response systems (McEwen, 2007; Wolkowitz *et al*., 2011b; Epel & Pranther, 2018). However, investigation of functional mechanisms involved was beyond the scope of the present study.

5.6 Moderating effects of selected polymorphisms in the serotonin transporter gene and tryptophan hydroxylase 2 gene on the association between acute stress and internalizing mental disorders

We determined the association between selected common polymorphisms within *SLC6A4* and *TPH2* and IMDs. In line with previous studies, we found no association between any of the selected *SLC6A4* polymorphisms and IMDs (Xiao *et al*., 2019; Culverhouse *et al*., 2018; Munafò *et al*., 2009a; Munafò *et al*, 2009b). The association between acute stress and IMDs was found to be significantly moderated by the *5-HTTLPR*/rs25531 *S-A-S-A* haplotype. Our finding that the interaction between stress and polymorphisms within the *SLC6A4* influences IMDs is in line with multiple previous studies (Conway, Slavich & Hammen, 2014; Sharpley *et al*., 2014; Karg, Burmeister, Shedden & Sen, 2011; Caspi *et al*., 2003), although they contradict with findings from a big meta-analysis (n = 38,802) that found no evidence of interaction between stress and *SLC6A4* polymorphisms on an IMD of depression among European-ancestry participants (Culverhouse *et al*., 2018). The lack of a significant association/interaction could be due to the complex nature of depression where several

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common variants exert small effects on IMDs (Plomin & Davis, 2009). However it should be noted that another bigger meta-analysis (n = 40,749) found evidence of interaction between stress and *SLC6A4* polymorphisms on depression (Karg, Burmeister, Shedden & Sen, 2011). It should however be noted that there is heterogeneity in terms of the type of the stressors and the rating scales for depression in both meta-analyses. There is need for more data with homogeneous measures of stress, rating scales for depression and diverse populations including Africans to make better deductions.

The lack of direct association between *SLC6A4* polymorphisms and IMDs in the present study could be due to a below threshold risk of IMDs in the absence of acute stress i.e. the presence of acute stress interacts with this underlying risk as per the diathesis-stress model (Monroe & Simons, 1991). The *5-HTTLPR SS* genotype alone was found to have no moderating effect on the association between acute stress and IMDs in the present study, highlighting the importance of both *5-HTTLPR* and rs25531 in the interaction. The rs25531 has been reported to influence *SLC6A4* transcription (Ehli *et al*, 2012; Hu *et al*., 2006) and thus analyzing *5-HTTLPR* together with rs25531 may present a better reflection of the transcriptional efficiency of *SLC6A4* than analyzing *5-HTTLPR* alone. Indeed, considering *5-HTTLPR* without rs25531 does not separate the *L-G* from the *L-A* haplotypes, this could result in individuals being incorrectly classified as low risk (Hankin, Jenness, Abela & Smolen, 2011). Future studies should endeavor to analyze *5-HTTLPR* together with rs25531 and functional studies should seek to quantify the risk conferred by each individual haplotype.

The *5-HTTLPR SS* genotype and rs25531 have been associated with reduced expression of *SLC6A4*. If lower *SLC6A4* expression levels indeed interact with stress to increase the risk for IMDs, it is worthwhile to note that there are several factors that influence *SLC6A4* expression, including DNA methylation and RNA interference. These factors may moderate the stress-associated risk of developing an IMD by increasing genetic vulnerability load (Epel *et al*., 2018). Thus, efforts are required to better characterize *SLC6A4* transcription in order to unmask this underlying risk for IMDs.

A significant association between *TPH2* rs1843809 and IMDs was observed where the *TG* and *TT* genotypes were significantly associated with reduced risk for IMDs, compared to the *GG* genotype. TPH2 is the rate-limiting enzyme in the production of 5-HT, and the TPH2 isoform is expressed exclusively in 5-HT neurons in the brain (Walther *et al*., 2003). The functional

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relevance of rs1843809 is not certain. Being an intronic variant, it is less likely that it would have a marked effect on *TPH2* expression or TPH2 structure, as introns are spliced during mRNA processing. However, this SNP may still exert functional effects, as potential effects of intronic variants have been suggested (de Almeida *et al*., 2017; Cooper, 2010; Kleinjan & van Heyningen, 2005). In addition, rs1843809 is in LD with a missense rs142055199 SNP among the Luhya population. Both the Luhya and Baganda speak the same Niger-Congo language (Countries & their cultures, 2019) and East Africa populations that speak this class of language have been found to be genetically similar by principal components analysis on GWAS data (Unpublished results provided by Anne Stevenson & Elizabeth Atkinson). The rs142055199 is located in the zinc finger C3H1-type containing gene, which modulates interleukin-8 (IL-8) transcription (National Center for Biotechnology Information, 2019). Modulation of IL-8 would be of interest since IMDs have been associated with inflammatory processes (Wang *et al*., 2019; Hori & Kim, 2019; Musinguzi *et al*., 2018; Miller, Maletic & Raison, 2009).

In line with our hypothesis, acute stress significantly associated with IMDs and this association was moderated by vulnerability due to chronic stress and *5-HTTLPR*/rs25531 respectively. We also found an association between rs1843809 with IMDs, however found no moderating role of the SNP on the association between acute stress and IMDs was observed.

We also hypothesize that accelerated TL attrition does not confer risk to IMDs but is rather an outcome of the biochemical process that accompany IMDs. We further hypothesize that accelerated TL attrition is mediated via genetic vulnerability to shorter TL by *TERT* and *TERC*. We therefore updated our conceptual framework (Figure 5.2) from the earlier conceptual framework (Figures 5.1 and 5.2).

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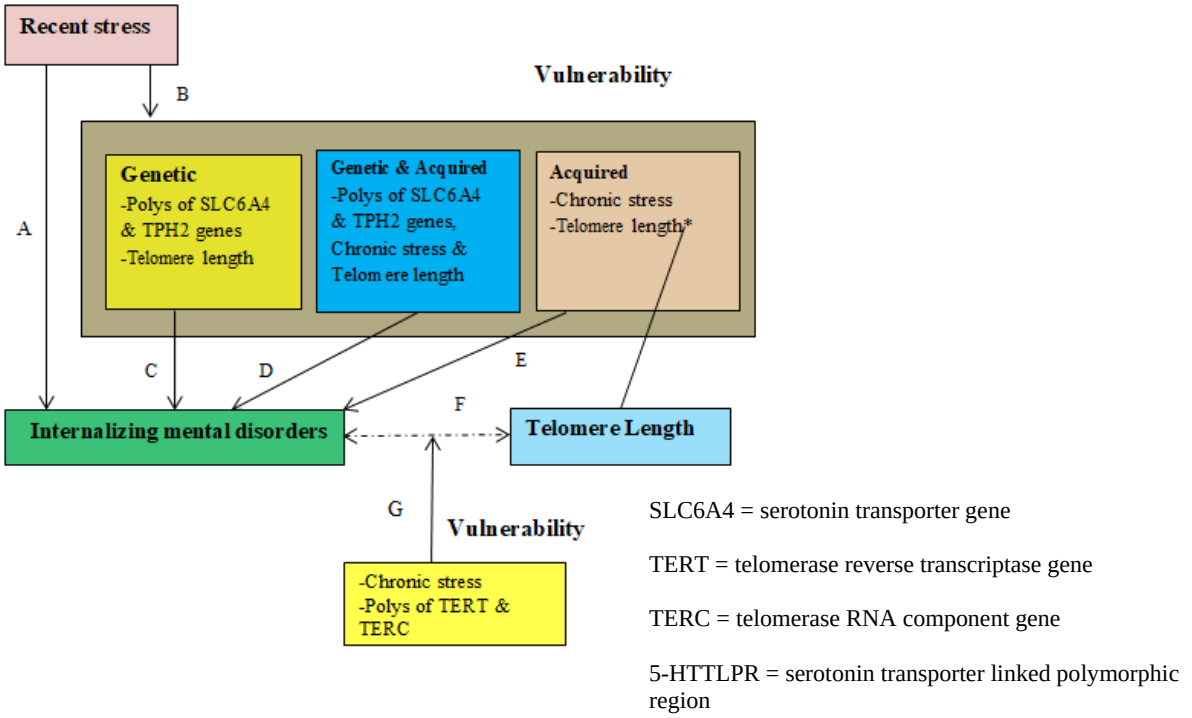

Figure 5.1: The original conceptual framework

The original conceptual framework depicts the serotonin transporter and the tryptophan hydroxylase 2 genes as the genetic risk (vulnerability) factors for internalizing mental disorders (IMDs) and chronic stress and telomere length as the acquired (environmental) vulnerability factors for IMDs. The framework depicts (dashed line) association between TL and IMDs whose nature is not unknown but hypothesized to be moderated by chronic stress and genetic vulnerability to shorter TL by telomerase reverse transcriptase gene (TERT) and telomerase RNA component gene (TERC).

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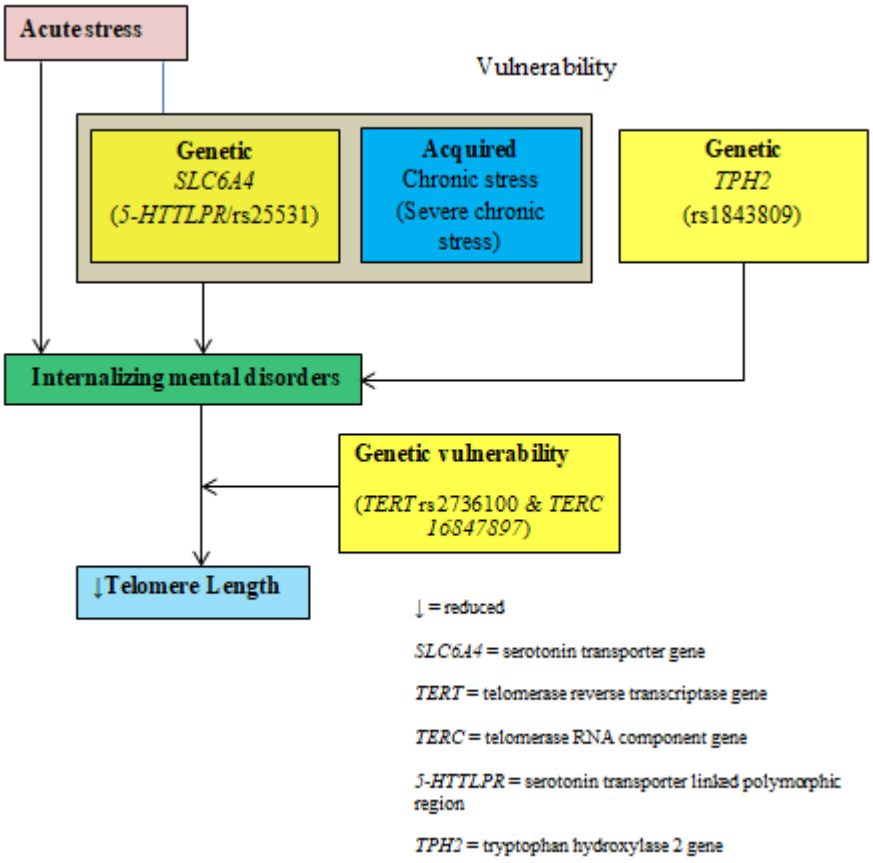

Figure 5.2: Proposed new conceptual framework depicting findings of the present study
*The new conceptual framework depicts both the serotonin transporter gene and the tryptophan hydroxylase 2 gene as the genetic risk (vulnerability) factors for internalizing mental disorders (IMDs). It excludes TL and depicts chronic stress as the acquired (environmental) vulnerability factors for IMDs. The framework also highlights reduced TL as an outcome of IMDs, an association that is not moderated by chronic stress but telomerase reverse transcriptase gene (*TERT*) rs2736100 and telomerase RNA component gene (*TERC*) rs16847897 single nucleotide polymorphisms.*

The present study provides evidence that the diathesis-stress model contributes to the etiology of IMDs. In line with the model, acute stress is associated with IMD caseness. Acute stress-associated risk for depression is in turn influenced by chronic stress and *5-HTTLPR*/rs25531 vulnerability factors. The mechanisms through which these vulnerability factors interact with acute stress to lead to IMDs are not well known. Understanding these mechanisms will make an important contribution towards the discovery of better drugs for managing IMDs. The direct

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association between *TPH2* rs1843809 with IMDs also presents *TPH2* as a candidate for vulnerability to IMDs. However, this association could not be influenced by acute stress since *TPH2* rs1843809 did not significantly moderate the association between acute stress and IMDs.

This study further provides evidence that IMD associated TL attrition is moderated by genetic variations in *TERT* and *TERC*. The mechanisms through which IMDs interact with *TERT* and *TERC* to influence TL still need to be elucidated. Our original conceptual framework has changed such that the bi-directional arrow between IMDs and TL has been replaced by a unidirectional arrow indicating the reduced TL is an outcome of IMDs, and TL is removed as a vulnerability factor for IMDs (Figure 5.2).

5.7 Limitations and strengths of the present study

This study has several limitations, which deserve mention. First, both acute and chronic stresses were measured using variables that were selected *a posteriori* because there is currently no locally adapted tool for assessing acute and chronic stress. As much as the variables used to generate the acute and chronic stress indices are known stressors in this population (Kinyanda *et al*., 2011a, b, 2013, 2017; Abbo *et al*, 2013), the lack of validation may limit generalizability of our findings to other settings. We recommend the local adaptation and validation of standardized assessment tools for acute and chronic stress for use in sub-Saharan settings, such as Uganda.

Second, we defined our cases as those individuals diagnosed with any depressive disorder or anxiety disorder or PTSD. The inclusion of PTSD (n = 60) in this sample is contentious, as the disorder has recently been excluded from the IMD category in the DSM-5 (American Psychiatric Association, 2013). This may have affected our findings. In order to elucidate the independent contribution of each disorder, we recommend that future studies investigate each IMD independently.

Third, both the duration and severity of IMDs have been shown to affect TL (Verhoeven *et al*., 2014; Lidqvist *et al*., 2015). We did not assess the duration of IMDs. However, we think that this may not have greatly affected our findings because disease severity was not significantly associated with TL in the present study. Future studies should, however, account for the duration of IMDs.

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Fourth, we also measured TL as relative TL using a previously published qPCR method (Cawthon, 2002), where mean telomere repeat copy number was normalized to a stably expressed single copy *HBG*, to control for differences in DNA quantity.

A comparison of TL measured as relative TL by qPCR (our measure of TL) with an absolute measure of TL (Southern blot analysis) has reported both methods to produce highly reproducible results as shown by r^2 values of greater than 0.9 for the correlations between results obtained by either method on two occasions (Aviv *et al*., 2011). In addition, the method we used to measure TL is well validated (Cawthon, 2002) and the methods used allowed control for PCR efficiency and both inter- and intra-plate variations (see section 5.5) using the GenEx software (GenEx User Guide, 2012). However, it is noteworthy that the qPCR method has a relatively high inter-assay coefficient of variation (6.45%) compared with Southern blots (1.74%) (Aviv *et al*., 2011) and this could affect comparison of TL measured by qPCR to those measured by Southern blots.

Fifth, the qPCR efficiencies were outside the optimal range of 90 – 110% (please see addendum E for the standard curves and the dissociation graphs) (Čepin, 2017). However, as would be expected, the gradient of the slopes for both TL repeats and the single copy reference gene, *HBG*, is negative i.e. the Ct value increases as the sample concentration decreases. Second, the Ct values and sample concentrations were moderately correlated for TL and strongly correlated for *HBG* with mean R^2 across the 22 plates of 0.485 and 0.808 respectively. In plotting our TL graphs, we observed several outlier points. These were predominantly at the highest and lowest concentrations in the serial dilution. A thorough explanation for this pattern is available (Čepin, 2017). Briefly, amplification efficiencies higher than 100% can be caused by polymerase inhibition, which may be due to excessive amounts of DNA, or common contaminants including heparin, hemoglobin, ethanol and phenol. Inhibition is more likely to occur in concentrated samples, increasing the number of amplification cycles required to reach the fluorescence threshold and depressing the efficiency plot slope. The ΔCt values between samples are thus smaller, resulting in amplification efficiency above 100%. Though polymerase inhibition is less of a problem in more dilute samples, the variability in the obtained Ct values can be higher due to the relatively low number of target molecules present in the sample. In such scenarios, the data obtained at the very highest and lowest concentrations should be excluded from the efficiency analyses. Given this reasoning, we chose to exclude outlier values from the efficiency plots, especially when these were at the extreme values of

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the dilution series. A similar approach was taken in the *HBG* analyses, though this was less frequently required. Though the rationale for this approach is clear, one consequence of this is that the range of concentration and Ct values included in the efficiency calculations is substantially reduced. Data preprocessing was performed using GenEx software. According to their documentation, they recommend constructing standard curves using 21 standards covering a concentration range of approximately 6 logs (GenEx User Guide, 2012). They further report that the PCR efficiencies calculated from standard curves with fewer standards and a lower spread of concentrations may be unreliable. Inspection of the graphs revealed better amplification for the *HBG* samples compared to the TL repeats. For this reason, we set the efficiency correction for TL and *HBG* data to 75% and 90% respectively. Though the amplification efficiencies are certainly a limitation in the analyses, our results did show good separation of TL and *HBG* dissociation curves and the expected inversely linear relationship between the log of the sample concentration and the obtained Ct values, giving us greater confidence in the validity of our results.

Sixth, we obtained the DNA from blood. As TL has been found to vary across different tissues (Demanelis *et al*., 2019), TL measured in blood may not be a true picture of TL in the brain. Nevertheless, whole blood TL has been reported to positively correlate with TL from other tissues (Demanelis *et al*., 2019) and thus can be used as a proxy for TL in other tissues such as the brain.

Seventh, we did not control for population stratification at analysis. The study participants belong to the Bagandan population group that has been found to be genetically homogeneous, given the low genetic variation 0.3 and 0.1% that has been explained by principal components 1 and 2 respectively (Gurdasani *et al*., 2019). There is, however, a possibility that some participants may not have been Bagandan even though they could speak Luganda (the main language of Baganda and the language into which study instruments were translated), indicating that we may have had a mildly admixed sample. Future studies should control for population stratification in order to circumvent confounding of results due to possible ancestry associated differences in genetic architecture.

Strengths of the present study

This study is the first to investigate genetic and environmental risk for IMDs among HIV+ children and adolescents in an African population. Although there were a number of

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limitations, this study also has a number of strengths. First, the study was carried out among a clinically well characterized study population, where IMDs were assessed using the DSM-5 referenced CASI-5 that was taken through a local adaptation process (Mpango *et al*., 2017) and whose psychometric properties in the Ugandan environment were determined and reported (Kinyanda *et al*., 2019).

Second, the study population represents an understudied population of African ancestry. There is a general paucity of data on genetic risk factors for IMDs and psychiatric disorders among African populations, which could affect the applicability of genetic risk factors discovered among populations of European descent to those of African descent. There is thus an urgent need for genomic studies among populations of African ancestry and the present study makes an effort towards closing this gap (van der Merwe *et al*., 2018; Peterson *et al*., 2019).

IMDs that arise in childhood and adolescence tend to persist into adulthood and have been reported to show a higher familial aggregation (Rapee, 2018; Wickramaratne & Weissman, 1998; Weissman *et al*., 1984). This suggests that childhood and adolescent onset IMDs could greatly be explained by genetic factors hence their importance in genetics studies.

5.9 Conclusions

In line with the diathesis-stress model, severe chronic stress and the *S-A-S-A* haplotype of *5-HTTLPR*/rs25531 are risk factors that interact with acute stress to increase the likelihood of the occurrence of IMDs among Ugandan HIV+ children and adolescents. The mechanisms through which stress interacts with vulnerability to lead to IMDs will offer insight into the etiology of IMDs. *TPH2* rs1843809 associated with IMDs where *TPH2* rs1843809 *TG* and *TT* genotypes were found to be protective against IMDs compared to *GG* genotype. Further studies are required to investigate the role of *TPH2* variations in IMDs.

Telomere length was longer among cases compared to controls at baseline; however, we found no significant association between IMDs and TL at 12 months highlighting that IMDs could potentially be responsible for our observations. We caution that other factors may have been responsible or could have contributed to this observation. These observations highlight accelerated reduction in TL as a potential outcome of IMDs but not a risk factor for IMDs. *TERT* rs2736100 and *TERC* rs16847897 were associated with lack of a statistically significant

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difference in TL between cases and controls after 12 months. Further studies are required to fully elucidate these mechanisms.

Future studies are required to understand how acute stress interacts with genetic polymorphisms to influence development of IMDs. Future studies should also endeavor to understand how IMDs interact with polymorphisms in *TERT* and *TERC* to influence TL attrition. Understanding the molecular etiology of IMDs is important for future efforts towards discovery of new drugs or putative drug targets for IMDs, as well as biomarkers for diagnosis.

IMDs are complex disorders where the gene-environment interactions that are responsible for behavioral disturbances involve a large number of genes, with each contributing to phenotypic variability to a very small extent (Plomin & Davis, 2009). GWASes and studies on polygenic risk scores may provide better insights into the risk underlying the development of IMDs. Furthermore, understanding how IMDs interact with genetic polymorphisms to influence TL may explain the accelerated aging due to IMDs and may elucidate the nature of the comorbidity observed between IMDs and metabolic diseases such as diabetes and cardiovascular disease, hence the need for further studies among adults to understand the relationship between IMDs, TL, and age-related diseases.

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6.0 References

Abbo, C., Kinyanda, E., Kizza, R. B., Levin, J., Ndyanabangi, S., & Stein, D. J. (2013). Prevalence, comorbidity and predictors of anxiety disorders in children and adolescents in rural north-eastern Uganda. *Child and Adolescent Psychiatry and Mental Health*, 7(1), 21.

Abebe, H., Shumet, S., Nassir, Z., Agidew, M., & Abebaw, D. (2019). Prevalence of depressive symptoms and associated factors among HIV-Positive youth attending ART follow-up in Addis Ababa, Ethiopia. *AIDS Research and Treatment*7.

Abrahin, O., Cortinhas-Alves, E. A., Vieira, R. P., & Guerreiro, J. F. (2019). Elite athletes have longer telomeres than sedentary subjects: A meta-analysis. *Experimental Gerontology*, 119, 138-145.

Adelman, R. D., Tmanova, L. L., Delgado, D., Dion, S., & Lachs, M. S. (2014). Caregiver burden: A clinical review. *Journal of the American Medical Association*, 311(10), 1052-1060.

Ahlers, K. P. (2016). *Living with HIV: A potential source of trauma in children and adolescents.* Unpublished MA thesis, University of Montana Missoula, Missoula, MT.

Ahmadi, N., Hajsadeghi, F., Mirshkarlo, H. B., Budoff, M., Yehuda, R., & Ebrahimi, R. (2011). Post-traumatic stress disorder, coronary atherosclerosis, and mortality. *The American Journal of Cardiology*, 108(1), 29-33.

Akena, D., Joska, J., Obuku, E. A., Amos, T., Musisi, S., & Stein, D. J. (2012). Comparing the accuracy of brief versus long depression screening instruments which have been validated in low and middle income countries: a systematic review. *BMC Psychiatry*, 12(1), 187.

Al Khaldi, R., Mojiminiyi, O., AlMulla, F., & Abdella, N. (2015). Associations of TERC single nucleotide polymorphisms with human leukocyte telomere length and the risk of type 2 diabetes mellitus. *PloS one*, 10(12), e0145721.

Alejos, B., Stella-Ascariz, N., Montejano, R., Rodriguez-Centeno, J., Schwimmer, C., Bernardino, J. I., ... & De Miguel, R. (2019). Determinants of blood telomere length in antiretroviral treatment-naïve HIV-positive participants enrolled in the NEAT 001/ANRS 143 clinical trial. *HIV Medicine*, 20(10), 691-698.

Stellenbosch University https://scholar.sun.ac.za

Alexeeff, S. E., Schaefer, C. A., Kvale, M. N., Shan, J., Blackburn, E. H., Risch, N., ... & Quesenberry, C. P. (2019). Telomere length and socioeconomic status at neighborhood and individual levels among 80,000 adults in the Genetic Epidemiology Research on Adult Health and Aging cohort. *Environmental Epidemiology*, 3(3), e049.

Ali, F. R., Vasiliou, S. A., Haddley, K., Paredes, U. M., Roberts, J. C., Miyajima, F., ... & Quinn, J. P. (2010). Combinatorial interaction between two human serotonin transporter gene variable number tandem repeats and their regulation by CTCF. *Journal of Neurochemistry*, 112(1), 296-306.

Allsopp, R. C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V., Futcher, A. B., ... & Harley, C. B. (1992). Telomere length predicts replicative capacity of human fibroblasts. *Proceedings of the National Academy of Sciences*, 89(21), 10114-10118.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (DSM-5®). Washington, DC: The Association.

Angold, A., Costello, E. J., Messer, S. C., & Pickles, A. (1995). Development of a short questionnaire for use in epidemiological studies of depression in children and adolescents. *International Journal of Methods in Psychiatric Research*, 5, 237–249.

Anttila, S., Viikki, M., Huuhka, K., Huuhka, M., Huhtala, H., Rontu, R., ... & Leinonen, E. (2009). TPH2 polymorphisms may modify clinical picture in treatment-resistant depression. *Neuroscience Letters*, 464(1), 43-46.

Arango, V., Underwood, M. D., Boldrini, M., Tamir, H., Kassir, S. A., Hsiung, S. C., ... & Mann, J. J. (2001). Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology*, 25(6), 892-903.

Aristizabal, M. J., Anreiter, I., Halldorsdottir, T., Odgers, C. L., McDade, T. W., Goldenberg, A., ... & O'Donnell, K. J. (2019). Biological embedding of experience: A primer on epigenetics. *Proceedings of the National Academy of Sciences*, 201820838.

Armanios, M. Y., Chen, J. J. L., Cogan, J. D., Alder, J. K., Ingersoll, R. G., Markin, C., ... & Lansdorp, P. M. (2007). Telomerase mutations in families with idiopathic pulmonary fibrosis. *New England Journal of Medicine*, 356(13), 1317-1326.

Armbruster, D., Moser, D. A., Strobel, A., Hensch, T., Kirschbaum, C., Lesch, K. P., & Brocke, B. (2009). Serotonin transporter gene variation and stressful life events impact processing of fear and anxiety. *International Journal of Neuropsychopharmacology*, 12(3), 393-401.

Stellenbosch University https://scholar.sun.ac.za

Artigas, F. (2013a). Serotonin receptors involved in antidepressant effects. *Pharmacology & Therapeutics*, 137(1), 119-131.

Artigas, F. (2013b). Future directions for serotonin and antidepressants. *ACS Chemical Neuroscience*, 4(1), 5-8.

Assary, E., Vincent, J. P., Keers, R., & Pluess, M. (2018). Gene-environment interaction and psychiatric disorders: Review and future directions. *Seminars in Cell & Developmental Biology*, 77, 133-143.

Atkinson, S. D., Prakash, A., Zhang, Q., Pangallo, B. A., Bangs, M. E., Emslie, G. J., & March, J. S. (2014). A double-blind efficacy and safety study of duloxetine flexible dosing in children and adolescents with major depressive disorder. *Journal of Child and Adolescent Psychopharmacology*, 24(4), 180-189.

Attanayake, V., McKay, R., Joffres, M., Singh, S., Burkle Jr, F., & Mills, E. (2009). Prevalence of mental disorders among children exposed to war: A systematic review of 7,920 children. *Medicine Conflict and Survival*, 25(1), 4-19.

Auld, E., Lin, J., Chang, E., Byanyima, P., Ayakaka, I., Musisi, E., ... & Huang, L. (2016). HIV infection is associated with shortened telomere length in Ugandans with suspected tuberculosis. *PloS one*, 11(9), e0163153.

Avetyan, D., Zakharyan, R., Petrek, M., & Arakelyan, A. (2019). Telomere shortening in blood leukocytes of patients with posttraumatic stress disorder. *Journal of Psychiatric Research*, 111, 83-88.

Aviv, A. (2012). Genetics of leukocyte telomere length and its role in atherosclerosis. *Mutatation Research*, 730, 68–74.

Aviv, A., Hunt, S. C., Lin, J., Cao, X., Kimura, M., & Blackburn, E. (2011). Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. *Nucleic Acids Research*, 39(20), e134-e134.

Baca-García, E., Vaquero, C., Diaz-Sastre, C., Saiz-Ruiz, J., Fernández-Piqueras, J., & de Leon, J. (2002). A gender-specific association between the serotonin transporter gene and suicide attempts. *Neuropsychopharmacology*, 26(5), 692.

Barrett, J. C. (2009). Haploview: Visualization and analysis of SNP genotype data. Cold Spring Harbor Protocols, 2009(10), pdb-ip71.

Bassuk, E. L., Richard, M. K., & Tsertsvadze, A. (2015). The prevalence of mental illness in homeless children: A systematic review and meta-analysis. *Journal of the American Academy of Child & Adolescent Psychiatry*, 54(2), 86-96.

Stellenbosch University https://scholar.sun.ac.za

Battersby, S., Ogilvie, A. D., Smith, C. A., Blackwood, D. H., Muir, W. J., Quinn, J. P., ... & Harmar, A. J. (1996). Structure of a variable number tandem repeat of the serotonin transporter gene and association with affective disorder. *Psychiatric Genetics*, 6(4), 177-181.

Beidel, D. C., Turner, S. M., Hamlin, K., & Morris, T. L. (2000). The Social Phobia and Anxiety Inventory for Children (SPAI-C): external and discriminative validity. *Behavior Therapy*, 31(1), 75-87.

Benke, K. S., Nivard, M. G., Velders, F. P., Walters, R. K., Pappa, I., Scheet, P. A., ... & Verhulst, F. C. (2014). A genome-wide association meta-analysis of preschool internalizing problems. *Journal of the American Academy of Child & Adolescent Psychiatry*, 53(6), 667-676.

Bernstein, A., Zvolensky, M. J., Feldner, M. T., Lewis, S. F., Fauber, A. L., Leen-Feldner, E. W., & Vujanovic, A. A. (2005). Anxiety sensitivity taxon and trauma: Discriminant associations for posttraumatic stress and panic symptomatology among young adults. *Depression and Anxiety*, 22(3), 138-149.

Betancourt, T., Scorza, P., Kanyanganzi, F., Fawzi, M. C. S., Sezibera, V., Cyamatare, F., ... & Kayiteshonga, Y. (2014). HIV and child mental health: a case-control study in Rwanda. *Pediatrics*, 134(2), e464.

Beusenberg, M., Orley, J. H., & World Health Organization. (1994). A User's guide to the self reporting questionnaire (SRQ) (No. WHO/MNH/PSF/94.8. Unpublished). Geneva: *World Health Organization*.

Bhatia, R., Hartman, C., Kallen, M. A., Graham, J., & Giordano, T. P. (2011). Persons newly diagnosed with HIV infection are at high risk for depression and poor linkage to care: results from the Steps Study. *AIDS and Behavior*, 15(6), 1161-1170.

Birleson, P. (1981). The validity of depressive disorder in childhood and the development of a self-rating scale: A research report. *Journal of Child Psychology and Psychiatry*, 22(1), 73-88.

Bisson, J. I., Cosgrove, S., Lewis, C., & Roberts, N. P. (2015). Post-traumatic stress disorder. *British Medical Jounral*, 351, h6161.

Blackburn, E. H., Greider, C. W., & Szostak, J. W. (2006). Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nature Medicine*, 12(10), 1133-1138.

Boscarino, J. A. (2006). Posttraumatic stress disorder and mortality among US Army veterans 30 years after military service. *Annals of Epidemiology*, 16(4), 248-256.

Stellenbosch University https://scholar.sun.ac.za

Boyes, M. E., & Cluver, L. D. (2015). Relationships between familial HIV/AIDS and symptoms of anxiety and depression: The mediating effect of bullying victimization in a prospective sample of South African children and adolescents. *Journal of Youth and Adolescence*, 44(4), 847-859.

Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G., ... & Henders, A. (2013). Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics*, 21(10), 1163.

Brown, R. C., Nugent, N. R., Hawn, S. E., Koenen, K. C., Miller, A., Amstadter, A. B., & Saxe, G. (2016). Predicting the transition from acute stress disorder to posttraumatic stress disorder in children with severe injuries. *Journal of Pediatric Health Care*, 30(6), 558-568.

Bui, E., Ohye, B., Palitz, S., Olliac, B., Goutaudier, N., Raynaud, J. P., ... & Stoddard Jr, F. J. (2014). Acute and chronic reactions to trauma in children and adolescents. *IACAPAP e-Textbook of Child and Adolescent Mental Health*. Geneva: International Association for Child and Adolescent Psychiatry and Allied Professions.

Bürgin, D., O'Donovan, A., d'Huart, D., di Gallo, A., Eckert, A., Fegert, J., ... & Boonmann, C. (2019). Adverse childhood experiences and telomere length: A look into the heterogeneity of findings. *Frontiers in Neuroscience*, 13, 490.

Cai, N., Bigdeli, T. B., Kretzschmar, W., Li, Y., Liang, J., Song, L., ... & Wang, G. (2015a). Sparse whole-genome sequencing identifies two loci for major depressive disorder. *Nature*, 523(7562), 588.

Cai, N., Chang, S., Li, Y., Li, Q., Hu, J., Liang, J., ... & Rivera, M. (2015b). Molecular signatures of major depression. *Current Biology*, 25(9), 1146-1156.

Campbell, M. C., & Tishkoff, S. A. (2008). African genetic diversity: Implications for human demographic history, modern human origins, and complex disease mapping. *Annual Review of Genomics and Human Genetics*, 9, 403-433.

Cao, C., Wang, L., Wang, R., Qing, Y., & Zhang, J. (2014). TPH2 genotype is associated with PTSD's avoidance symptoms in Chinese female earthquake survivors. *Psychiatric Genetics*, 24(6), 257-261.

Carkaci-Salli, N., Flanagan, J. M., Martz, M. K., Salli, U., Walther, D. J., Bader, M., & Vrana, K. E. (2006). Functional domains of human tryptophan hydroxylase 2 (hTPH2). *Journal of Biological Chemistry*, 281(38), 28105-28112.

Stellenbosch University https://scholar.sun.ac.za

Caspi, A., & Moffitt, T. E. (2006). Gene–environment interactions in psychiatry: Joining forces with neuroscience. *Nature Reviews Neuroscience*, 7(7), 583.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., ... & Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, 301(5631), 386-389.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic acids Research*, 30(10), e47-e47.

Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Research*, 37(3), e21-e21.

Cervilla, J. A., Molina, E., Rivera, M., Torres-Gonzalez, F., Bellon, J. A., Moreno, B., ... & Nazareth, I. (2007). The risk for depression conferred by stressful life events is modified by variation at the serotonin transporter 5HTTLPR genotype: Evidence from the Spanish PREDICT-Gene cohort. *Molecular Psychiatry*, 12(8), 748.

Čepin, U. 2017. Understanding qPCR Efficiency and Why It Can Exceed 100%. BioSistemika. https://biosistemika.com/blog/qpcr-efficiency-over-100/ (accessed 5 February 2020).

Cesare, A. J., and Reddel, R. R. (2010). Alternative lengthening of telomeres: Models, mechanisms and implications. *Nature Reviews Genetics*, 11 (5), 319.

Chang, S. C., Prescott, J., De Vivo, I., Kraft, P., & Okereke, O. I. (2018). Polygenic risk score of shorter telomere length and risk of depression and anxiety in women. *Journal of Psychiatric Research*, 103, 182-188.

Charney, D. S., & Manji, H. K. (2004). Life stress, genes, and depression: Multiple pathways lead to increased risk and new opportunities for intervention. *Science's STKE: Signal Transduction Knowledge Environment*, 2004(225), re5.

Chen, J. L., & Greider, C. W. (2003). Determinants in mammalian telomerase RNA that mediate enzyme processivity and cross-species incompatibility. *The EMBO Journal*, 22(2), 304-314.

Chen, S. H., Epel, E. S., Mellon, S. H., Lin, J., Reus, V. I., Rosser, R., ... & Wolkowitz, O. M. (2014). Adverse childhood experiences and leukocyte telomere maintenance in depressed and healthy adults. *Journal of Affective Disorders*, 169, 86-90.

Chen, X., Zeng, C., Gong, C., Zhang, L., Wan, Y., Tao, F., & Sun, Y. (2019). Associations between early life parent-child separation and shortened telomere length and psychopathological outcomes during adolescence. *Psychoneuroendocrinology*, 103, 195-202.

Stellenbosch University https://scholar.sun.ac.za

Cherkas, L. F., Hunkin, J. L., Kato, B. S., Richards, J. B., Gardner, J. P., Surdulescu, G. L., et al. (2008). The association between physical activity in leisure time and leukocyte telomere length. *Archives of Internal Medicine*. 168 (2), 154–158.

Chida, Y., & Vedhara, K. (2009). Adverse psychosocial factors predict poorer prognosis in HIV disease: A meta-analytic review of prospective investigations. *Brain, Behavior, and Immunity*, 23(4), 434-445.

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., ... & Egger, M. (2018). Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: A systematic review and network meta-analysis. *Focus*, 16(4), 420-429.

Cipriani, A., Zhou, X., Del Giovane, C., Hetrick, S. E., Qin, B., Whittington, C., ... & Cuijpers, P. (2016). Comparative efficacy and tolerability of antidepressants for major depressive disorder in children and adolescents: A network meta-analysis. *The Lancet*, 388(10047), 881-890.

Clarke, H., Flint, J., Attwood, A. S., & Munafo, M. R. (2010). Association of the 5-HTTLPR genotype and unipolar depression: A meta-analysis. *Psychological Medicine*, 40(11), 1767-1778.

Clarke, L., Fairley, S., Zheng-Bradley, X., Streeter, I., Perry, E., Lowy, E., ... & Flicek, P. (2017). The international Genome sample resource (IGSR): A worldwide collection of genome variation incorporating the 1000 Genomes Project data. *Nucleic Acids Research*, 45(D1), D854-D859.

Cluver, L. D., Orkin, M., Gardner, F., & Boyes, M. E. (2012). Persisting mental health problems among AIDS-orphaned children in South Africa. *Journal of Child Psychology and Psychiatry*, 53(4), 363-370.

Codd, V., Mangino, M., van der Harst, P., Braund, P. S., Kaiser, M., Beveridge, A. J., ... & Zhai, G. (2010). Common variants near TERC are associated with mean telomere length. *Nature Genetics*, 42(3), 197.

Codd, V., Nelson, C. P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J. L., ... & Broer, L. (2013). Identification of seven loci affecting mean telomere length and their association with disease. *Nature Genetics*, 45(4), 422.

Cohen, B. E., Edmondson, D., & Kronish, I. M. (2015). State of the art review: Depression, stress, anxiety, and cardiovascular disease. *American Journal of Hypertension*, 28(11), 1295-1302.

Stellenbosch University https://scholar.sun.ac.za

Cohen, S., Murphy, M. L., & Prather, A. A. (2019). Ten surprising facts about stressful life events and disease risk. *Annual Review of Psychology*, 70, 577-597.

Colton, C. W., & Manderscheid, R. W. (2006). Congruencies in increased mortality rates, years of potential life lost, and causes of death among public mental health clients in eight states. *Preventing Chronic Disease*, 3(2), A42.

Compton, W. M., Thomas, Y. F., Stinson, F. S., & Grant, B. F. (2007). Prevalence, correlates, disability, and comorbidity of DSM-IV drug abuse and dependence in the United States: Results from the national epidemiologic survey on alcohol and related conditions. *Archives of General Psychiatry*, 64(5), 566-576.

Conway, C. C., Slavich, G. M., & Hammen, C. (2014). Daily stress reactivity and serotonin transporter gene (5-HTTLPR) variation: Internalizing responses to everyday stress as a possible transdiagnostic phenotype. *Biology of Mood & Anxiety Disorders*, 4(1), 2.

Conway, K. P., Compton, W., Stinson, F. S., & Grant, B. F. (2006). Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Journal of Clinical Psychiatry*, 67(2), 247-257.

Cooper, D. N. (2010). Functional intronic polymorphisms: buried treasure awaiting discovery within our genes. 284-8.

Copeland, W. E., Keeler, G., Angold, A., & Costello, E. J. (2007). Traumatic events and posttraumatic stress in childhood. *Archives of General Psychiatry*, 64(5), 577-584.

Countries and their cultures. (2019). The Baganda. Retrieved from: https://www.everyculture.com/wc/Tajikistan-to-Zimbabwe/Baganda.html.

Côté, H. C., & Hsieh, A. Y. (2018). Leukocyte telomere length in HIV infection: A marker of persistent immune aging or transient immune reconstitution? *The Journal of Infectious Diseases*, 218(10), 1521-1522.

Cuijpers, P., & Smit, F. (2002). Excess mortality in depression: A meta-analysis of community studies. *Journal of Affective Disorders*, 72(3), 227-236.

Culverhouse, R. C., Saccone, N. L., Horton, A. C., Ma, Y., Anstey, K. J., Banaschewski, T., ... & Goldman, N. (2018). Collaborative meta-analysis finds no evidence of a strong interaction between stress and 5-HTTLPR genotype contributing to the development of depression. *Molecular Psychiatry*, 23(1), 133.

Czarny, P., Wigner, P., Galecki, P., & Sliwinski, T. (2018). The interplay between inflammation, oxidative stress, DNA damage, DNA repair and mitochondrial

Stellenbosch University https://scholar.sun.ac.za

dysfunction in depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 80, 309-321.

Dai, W., Liu, A., Kaminga, A. C., Deng, J., Lai, Z., & Wen, S. W. (2018). Prevalence of posttraumatic stress disorder among children and adolescents following road traffic accidents: A meta-analysis. *The Canadian Journal of Psychiatry*, 63(12), 798-808.

Dalvie, S., Koen, N., Duncan, L., Abbo, C., Akena, D., Atwoli, L., ... & Mall, S. (2015). Large scale genetic research on neuropsychiatric disorders in African populations is needed. *EBioMedicine*, 2(10), 1259-1261.

Damjanovic, A. K., Yang, Y., Glaser, R., Kiecolt-Glaser, J. K., Nguyen, H., Laskowski, B., ... & Weng, N. P. (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *The Journal of Immunology*, 179(6), 4249-4254.

Darrow, S. M., Verhoeven, J. E., Révész, D., Lindqvist, D., Penninx, B. W., Delucchi, K. L., ... & Mathews, C. A. (2016). The association between psychiatric disorders and telomere length: a meta-analysis involving 14,827 persons. *Psychosomatic Medicine*, 78(7), 776.

Davey, C. G., Yücel, M., & Allen, N. B. (2008). The emergence of depression in adolescence: Development of the prefrontal cortex and the representation of reward. *Neuroscience & Biobehavioral Reviews*, 32(1), 1-19.

Davies, M. N., Verdi, S., Burri, A., Trzaskowski, M., Lee, M., Hettema, J. M., ... & Spector, T. D. (2015). Generalised anxiety disorder–a twin study of genetic architecture, genome-wide association and differential gene expression. *PLoS One*, 10(8), e0134865.

de Almeida, R. M., Tavares, J., Martins, S., Carvalho, T., Enguita, F. J., Brito, D., ... & Lopes, L. R. (2017). Whole gene sequencing identifies deep-intronic variants with potential functional impact in patients with hypertrophic cardiomyopathy. *PloS One*, 12(8), e0182946.

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, 6(6), 463.

de Lara, C. L., Brezo, J., Rouleau, G., Lesage, A., Dumont, M., Alda, M., ... & Turecki, G. (2007). Effect of tryptophan hydroxylase-2 gene variants on suicide risk in major depression. *Biological Psychiatry*, 62(1), 72-80.

de Lijster, J. M., Dierckx, B., Utens, E. M., Verhulst, F. C., Zieldorff, C., Dieleman, G. C., & Legerstee, J. S. (2017). The age of onset of anxiety disorders: A meta-

Stellenbosch University https://scholar.sun.ac.za

analysis. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie*, 62(4), 237.

Demanelis, K., Jasmine, F., Chen, L. S., Chernoff, M., Tong, L., Shinkle, J., ... & Kim-Hellmuth, S. (2019). Determinants of telomere length across human tissues. *bioRxiv*, 793406.

Dieleman, G. C., Huizink, A. C., Tulen, J. H., Utens, E. M., Creemers, H. E., van der Ende, J., & Verhulst, F. C. (2015). Alterations in HPA-axis and autonomic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis.

Ding, D., Xi, P., Zhou, J., Wang, M., & Cong, Y. S. (2013). Human telomerase reverse transcriptase regulates MMP expression independently of telomerase activity via NF-κB-dependent transcription. *The FASEB Journal*, 27(11), 4375-4383.

Domschke, K., & Maron, E. (2013). Genetic factors in anxiety disorders. *Modern Trends in Pharmacopsychiatry*, 29, 24-46.

Douillard-Guilloux, G., Guilloux, J. P., Lewis, D. A., & Sibille, E. (2013). Anticipated brain molecular aging in major depression. *The American Journal of Geriatric Psychiatry*, 21(5), 450-460.

Drury, S. S., Shachet, A., Brett, Z. H., Wren, M., Esteves, K., Shirtcliff, E. A., ... & Theall, K. P. (2014). Growing up or growing old? Cellular aging linked with testosterone reactivity to stress in youth. *The American Journal of the Medical Sciences*, 348(2), 92-100.

Drury, S. S., Theall, K., Gleason, M. M., Smyke, A. T., De Vivo, I., Wong, J. Y. Y., ... & Nelson, C. A. (2012). Telomere length and early severe social deprivation: linking early adversity and cellular aging. *Molecular Psychiatry*, 17(7), 719.

Druss, B. G., Zhao, L., Von Esenwein, S., Morrato, E. H., & Marcus, S. C. (2011). Understanding excess mortality in persons with mental illness: 17-year follow up of a nationally representative US survey. *Medical Care*, 599-604.

Ehlers, A., Mayou, R. A., & Bryant, B. (2003). Cognitive predictors of posttraumatic stress disorder in children: Results of a prospective longitudinal study. *Behaviour Research and Therapy*, 41(1), 1-10.

Ehli, E. A., Hu, Y., Lengyel-Nelson, T., Hudziak, J. J., Davies, G. E. (2012). Identification and functional characterization of three novel alleles for the serotonin transporter-linked polymorphic region. *Molecular Psychiatry*, 17, 185–192.

Emslie, G. J., Prakash, A., Zhang, Q., Pangallo, B. A., Bangs, M. E., & March, J. S. (2014). A double-blind efficacy and safety study of duloxetine fixed doses in children and

Stellenbosch University https://scholar.sun.ac.za

adolescents with major depressive disorder. *Journal of Child and Adolescent Psychopharmacology*, 24(4), 170-179.

Epel, E. S., & Lithgow, G. J. (2014). Stress biology and aging mechanisms: toward understanding the deep connection between adaptation to stress and longevity. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 69(Suppl_1), S10-S16.

Epel, E. S., & Prather, A. A. (2018). Stress, telomeres, and psychopathology: toward a deeper understanding of a triad of early aging. *Annual Review of Clinical Psychology*, 14, 371-397.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., & Cawthon, R. M. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences*, 101(49), 17312-17315.

Epel, E. S., Merkin, S. S., Cawthon, R., Blackburn, E. H., Adler, N. E., Pletcher, M. J., & Seeman, T. E. (2009). The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging (Albany NY)*, 1(1), 81.

Ernst, C., Mechawar, N., & Turecki, G. (2009). Suicide neurobiology. *Progress in Neurobiology*, 89(4), 315-333.

Esteves, K. C., Jones, C. W., Wade, M., Callerame, K., Smith, A. K., Theall, K. P., & Drury, S. S. (2019). Adverse childhood experiences: Implications for offspring telomere length and psychopathology. *American Journal of Psychiatry*, appiajp201918030335.

Evans, G. W., Li, D., & Whipple, S. S. (2013). Cumulative risk and child development. *Psychological Bulletin*, 139(6), 1342.

Ezpeleta, L., Keeler, G., Erkanli, A., Costello, E. J., & Angold, A. (2001). Epidemiology of psychiatric disability in childhood and adolescence. *The Journal of Child Psychology and Psychiatry and Allied Disciplines*, 42(7), 901-914.

Faulstich, M. E., Carey, M. P., Ruggiero, L et al. (1986). Assessment of depression in childhood and adolescence: An evaluation of the Center for Epidemiological Studies Depression Scale for Children (CES-DC). *American Journal of Psychiatry*; 143(8):1024–1027.

Fergusson, D. M., & Mullen, P. E., (1999). Childhood sexual abuse: An evidence based perspective. *Developmental Clinical Psychology and Psychiatry Series*, 40. Thousand Oaks: Sage Publications, Inc.

Forero, D. A., González-Giraldo, Y., López-Quintero, C., Castro-Vega, L. J., Barreto, G. E., & Perry, G. (2016). Meta-analysis of telomere length in Alzheimer's disease. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 71(8), 1069-1073.

Stellenbosch University https://scholar.sun.ac.za

Frenck, R. W., Blackburn, E. H., & Shannon, K. M. (1998). The rate of telomere sequence loss in human leukocytes varies with age. *Proceedings of the National Academy of Sciences*, 95(10), 5607-5610.

Frewen, P., Zhu, J., & Lanius, R. (2019). Lifetime traumatic stressors and adverse childhood experiences uniquely predict concurrent PTSD, complex PTSD, and dissociative subtype of PTSD symptoms whereas recent adult non-traumatic stressors do not: Results from an online survey study. *European Journal of Psychotraumatology*, 10(1), 1606625.

Fuchs, E., & Flügge, G. (2006). Experimental animal models for the simulation of depression and anxiety. *Dialogues in Clinical Neuroscience*, 8(3), 323.

Fullerton, C. S., Ursano, R. J., & Wang, L. (2004). Acute stress disorder, posttraumatic stress disorder, and depression in disaster or rescue workers. *American Journal of Psychiatry*, 161(8), 1370-1376.

Furlong, R. A., Ho, L., Walsh, C., Rubinsztein, J. S., Jain, S., Paykel, E. S., ... & Rubinsztein, D. C. (1998). Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. *American Journal of Medical Genetics*, 81(1), 58-63.

Furmark, T., Marteinsdottir, I., Frick, A., Heurling, K., Tillfors, M., Appel, L., ... & Eriksson, E. (2016). Serotonin synthesis rate and the tryptophan hydroxylase-2: G-703T polymorphism in social anxiety disorder. *Journal of Psychopharmacology*, 30(10), 1028-1035.

Gadow KD, Sprafkin J (1999) Youth's Inventory-4 (YI-4). Stony Brook, NY: Checkmate Plus.

Gadow, K. D., Angelidou, K., Chernoff, M., Williams, P. L., Heston, J., Hodge, J., & Nachman, S. (2012). Longitudinal study of emerging mental health concerns in youth perinatally infected with HIV and peer comparisons. *Journal of Developmental and Behavioral Pediatrics*, 33(6), 456.

Gadow, K. D., Chernoff, M., Williams, P. L., Brouwers, P., Morse, E., Heston, J., ... & Nachman, S. (2010). Co-occuring psychiatric symptoms in children perinatally infected with HIV and peer comparison sample. *Journal of Developmental and Behavioral Pediatrics*, 31(2), 116.

Gadow. K.D., & Sprafkin .J. (2013) Child and Adolescent Symptom Inventory-5 manual. Stony Brook. NY. Checkmate Plus. https ://www. check matep lus.com/produ ct/casi5 .htm. Accessed June 29 2019.

Stellenbosch University https://scholar.sun.ac.za

Gao, J., Pan, Z., Jiao, Z., Li, F., Zhao, G., Wei, Q., ... & Evangelou, E. (2012). TPH2 gene polymorphisms and major depression–a meta-analysis. *PloS one*, 7(5), e36721.

Garcia-Rizo, C., Fernandez-Egea, E., Miller, B. J., Oliveira, C., Justicia, A., Griffith, J. K., ... & Kirkpatrick, B. (2013). Abnormal glucose tolerance, white blood cell count, and telomere length in newly diagnosed, antidepressant-naive patients with depression. *Brain, Behavior, and Immunity*, 28, 49-53.

Gardner, M. J., Thomas, H. J., & Erskine, H. E. (2019). The association between five forms of child maltreatment and depressive and anxiety disorders: A systematic review and meta-analysis. *Child Abuse & Neglect*, 96, 104082.

Gassó, P., Rodríguez, N., Boloc, D., Blázquez, A., Torres, T., Gortat, A., ... & Lázaro, L. (2017). Association of regulatory TPH2 polymorphisms with higher reduction in depressive symptoms in children and adolescents treated with fluoxetine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 77, 236-240.

Gelernter, J., Sun, N., Polimanti, R., Pietrzak, R., Levey, D. F., Bryois, J., ... & Aslan, M. (2019). Genome-wide association study of post-traumatic stress disorder reexperiencing symptoms in> 165,000 US veterans. *Nature Neuroscience*, 22(9), 1394-1401.

GenEx User Guide. 2012. Multid Analyses AB. https://www.gene-quantification.de/genex-user-guide.pdf (accessed 19 February 2020).

Gianesin, K., Noguera-Julian, A., Zanchetta, M., Del Bianco, P., Petrara, M. R., Freguja, R., ... & Giaquinto, C. (2016). Premature aging and immune senescence in HIV-infected children. *AIDS (London, England)*, 30(9), 1363.

Goçi, A. U., Agani, F., Blyta, A., Hoxha, B., Haxhibeqiri, S., Haxhibeqiri, V., ... & Dzubur-Kulenovic, A. (2019). Role of the Allelic Variation in the 5-Hydroxytryptamine Receptor 1A (HTR1A) and the Tryptophan Hydroxylase 2 (TPH2) Genes in the Development of PTSD. *Psychiatria Danubina*, 31(2), 256-262.

Goenjian, A. K., Bailey, J. N., Walling, D. P., Steinberg, A. M., Schmidt, D., Dandekar, U., & Noble, E. P. (2012). Association of TPH1, TPH2, and 5HTTLPR with PTSD and depressive symptoms. *Journal of Affective Disorders*, 140(3), 244-252.

Gonzalez-Serna, A., Ajaykumar, A., Gadawski, I., Muñoz-Fernández, M. A., Hayashi, K., Harrigan, P. R., & Côté, H. C. (2017). Rapid decrease in peripheral blood mononucleated cell telomere length after HIV seroconversion, but not HCV seroconversion. *Journal of Acquired Immune Deficiency Syndromes*, 76(1), e29.

Stellenbosch University https://scholar.sun.ac.za

Gotlib, I. H., LeMoult, J., Colich, N. L., Foland-Ross, L. C., Hallmayer, J., Joormann, J., ... & Wolkowitz, O. M. (2015). Telomere length and cortisol reactivity in children of depressed mothers. *Molecular Psychiatry*, 20(5), 615.

Gressier, F., Calati, R., Balestri, M., Marsano, A., Alberti, S., Antypa, N., & Serretti, A. (2013). The 5-HTTLPR polymorphism and posttraumatic stress disorder: A meta-analysis. *Journal of Traumatic Stress*, 26(6), 645-653.

Grillon, C., Duncko, R., Covington, M. F., Kopperman, L., & Kling, M. A. (2007). Acute stress potentiates anxiety in humans. *Biological Psychiatry*, 62(10), 1183-1186.

Guo, J., Yang, Y., Huang, P., Jiang, X., Guo, M., Liu, Z., & Pan, J. (2019). An associated research for genetic polymorphism of 5-HTTLPR with post-traumatic stress disorder. *Journal of Behavioral and Brain Science*, 9(01), 1.

Gurdasani, D., Carstensen, T., Fatumo, S., Chen, G., Franklin, C. S., Prado-Martinez, J., ... & Mathieson, I. (2019). Uganda Genome Resource Enables Insights into Population History and Genomic Discovery in Africa. *Cell*, 179(4), 984-1002.

Gurdasani, D., Carstensen, T., Tekola-Ayele, F., Pagani, L., Tachmazidou, I., Hatzikotoulas, K., ... & Ritchie, G. R. (2015). The African genome variation project shapes medical genetics in Africa. *Nature*, 517(7534), 327.

Haberstick, B. C., Boardman, J. D., Wagner, B., Smolen, A., Hewitt, J. K., Killeya-Jones, L. A., ... & Siegler, I. C. (2016). Depression, stressful life events, and the impact of variation in the serotonin transporter: Findings from the National Longitudinal Study of Adolescent to Adult Health (Add Health). *PloS one*, 11(3), e0148373.

Haddley, K., Bubb, V. J., Breen, G., Parades-Esquivel, U. M., & Quinn, J. P. (2011). Behavioural genetics of the serotonin transporter. In *Behavioral Neurogenetics* (pp. 503-535). Berlin, Heidelberg: Springer.

Hall, K. S., Beckham, J. C., Bosworth, H. B., Sloane, R., Pieper, C. F., & Morey, M. C. (2014). PTSD is negatively associated with physical performance and physical function in older overweight military Veterans. *Journal of Rehabilitation Research and Development*, 51(2), 285.

Hammen, C., & Rudolph, K. D. (2003). "Childhood mood disorders," in E. J. Mash & R. A. Barkley (eds.), *Child psychopathology* (2nd ed., pp. 233–278). New York, NY: Guilford Press.

Hammoud, D. A., Endres, C. J., Hammond, E., Uzuner, O., Brown, A., Nath, A., ... & Pomper, M. G. (2010). Imaging serotonergic transmission with [11C] DASB-PET in depressed and non-depressed patients infected with HIV. *Neuroimage*, 49(3), 2588-2595.

Stellenbosch University https://scholar.sun.ac.za

Hankin, B. L., Jenness, J., Abela, J. R., & Smolen, A. (2011). Interaction of 5-HTTLPR and idiographic stressors predicts prospective depressive symptoms specifically among youth in a multiwave design. *Journal of Clinical Child & Adolescent Psychology*, 40(4), 572-585.

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., ... & Weinberger, D. R. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science*, 297(5580), 400-403.

Hasegawa, H., & Nakamura, K. (2010). Tryptophan hydroxylase and serotonin synthesis regulation. In *Handbook of Behavioral Neuroscience* (Vol. 21, pp. 183-202). Oxford, UK: Elsevier.

Haycock, P. C., Heydon, E. E., Kaptoge, S., Butterworth, A. S., Thompson, A., & Willeit, P. (2014). Leucocyte telomere length and risk of cardiovascular disease: Systematic review and meta-analysis. *British Medical Journal*, 349, g4227.

Heils, A., Mössner, R., & Lesch, K. P. (1997). The human serotonin transporter gene polymorphism-basic research and clinical implications. *Journal of Neural Transmission*, 104(10), 1005-1014.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., & Lesch, K. P. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*, 66(6), 2621-2624.

Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D., ... & Mann, K. (2005). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience*, 8(1), 20.

Heo, M., Murphy, C. F., Fontaine, K. R., Bruce, M. L., & Alexopoulos, G. S. (2008). Population projection of US adults with lifetime experience of depressive disorder by age and sex from year 2005 to 2050. *International Journal of Geriatric Psychiatry*, 23(12), 1266-1270.

Herres, J., Shearer, A., Kodish, T., Kim, B., Wang, S. B., & Diamond, G. S. (2019). Differences in suicide risk severity among suicidal youth with anxiety disorders. *Crisis*, 40(5), 333-339.

Hettema, J. M., Neale, M. C., & Kendler, K. S. (2001). A review and meta-analysis of the genetic epidemiology of anxiety disorders. *American Journal of Psychiatry*, 158(10), 1568-1578.

Stellenbosch University https://scholar.sun.ac.za

Hettema, J. M., Prescott, C. A., Myers, J. M., Neale, M. C., & Kendler, K. S. (2005). The structure of genetic and environmental risk factors for anxiety disorders in men and women. *Archives of General Psychiatry*, 62(2), 182-189.

Heuser, I. (2002). Depression, endocrinologically a syndrome of premature aging? *Mauritas*, 41, 19-23.

Hoen, P. W., de Jonge, P., Na, B. Y., Farzaneh-Far, R., Epel, E., Lin, J., ... & Whooley, M. A. (2011). Depression and leukocyte telomere length in patients with coronary heart disease: Data from the Heart and Soul Study. *Psychosomatic Medicine*, 73(7), 541.

Hori, H., & Kim, Y. (2019). Inflammation and post-traumatic stress disorder. *Psychiatry and Clinical Neurosciences*, 73(4), 143-153.

Houben, J. M., Moonen, H. J., van Schooten, F. J., & Hageman, G. J. (2008). Telomere length assessment: Biomarker of chronic oxidative stress? *Free Radical Biology and Medicine*, 44(3), 235-246.

Howard, D. M., Adams, M. J., Shirali, M., Clarke, T. K., Marioni, R. E., Davies, G., ... & Wigmore, E. M. (2018). Genome-wide association study of depression phenotypes in UK Biobank identifies variants in excitatory synaptic pathways. *Nature Communications*, 9(1), 1470.

Hu, X. Z., Lipsky, R. H., Zhu, G., Akhtar, L. A., Taubman, J., Greenberg, B. D., ... & Murphy, D. L. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *The American Journal of Human Genetics*, 78(5), 815-826.

Hukezalie, K. R., Thumati, N. R., Coˆte, H. C., & Wong, J. M. (2012). In vitro and ex vivo inhibition of human telomerase by anti-HIV nucleoside reverse transcriptase inhibitors (NRTIs) but not by non-NRTIs. *PLoS One*, 7(11), e47505.

Hyde, C. L., Nagle, M. W., Tian, C., Chen, X., Paciga, S. A., Wendland, J. R., ... & Winslow, A. R. (2016). Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. *Nature Genetics*, 48(9), 1031.

Ingman, M., Kaessmann, H., Pääbo, S., & Gyllensten, U. (2000). Mitochondrial genome variation and the origin of modern humans. *Nature*, 408(6813), 708.

Ironson, G., O'cleirigh, C., Fletcher, M. A., Laurenceau, J. P., Balbin, E., Klimas, N., ... & Solomon, G. (2005). Psychosocial factors predict CD4 and viral load change in men and women with human immunodeficiency virus in the era of highly active antiretroviral treatment. *Psychosomatic Medicine*, 67(6), 1013.

Stellenbosch University https://scholar.sun.ac.za

Iurescia, S., Rinaldi, M., Pilotto, A., Paroni, G., De Dominicis, E., Gravina, C., Urbano, M., Borroni, B., et al. (2012). Identification and functional characterization of novel functional alleles in the promoter region of the serotonin transporter gene. *Neurological Sciences* 33: S401.

Jacob, J., Ferri, A. L., Milton, C., Prin, F., Pla, P., Lin, W., ... & Briscoe, J. (2007). Transcriptional repression coordinates the temporal switch from motor to serotonergic neurogenesis. *Nature Neuroscience*, 10(11), 1433.

Jakubovski, E., Johnson, J. A., Nasir, M., Müller-Vahl, K., & Bloch, M. H. (2019). Systematic review and meta-analysis: Dose–response curve of SSRIs and SNRIs in anxiety disorders. *Depression and Anxiety*, 36(3), 198-212.

Jawahar, M. C., Toben, C. G., & Baune, B. T. (2019). Gene-environment interactions and epigenetic mechanisms in depression. In Neurobiology of Depression (pp. 17-25). Oxford, UK: Academic Press.

Jenkins, R., Baingana, F., Ahmad, R., McDaid, D., & Atun, R. (2011). Social, economic, human rights and political challenges to global mental health. *Mental Health in Family Medicine*, 8(2), 87.

Stellenbosch University https://scholar.sun.ac.za

Jenness, J. L., Jager-Hyman, S., Heleniak, C., Beck, A. T., Sheridan, M. A., & McLaughlin, K. A. (2016). Catastrophizing, rumination, and reappraisal prospectively predict adolescent PTSD symptom onset following a terrorist attack. *Depression and Anxiety*, 33(11), 1039-1047.

Jones, A. M., Beggs, A. D., Carvajal-Carmona, L., Farrington, S., Tenesa, A., Walker, M., ... & Bertagnolli, M. (2012). TERC polymorphisms are associated both with susceptibility to colorectal cancer and with longer telomeres. *Gut*, 61(2), 248-254.

Kalungi, A., Seedat, S., Hemmings, S. M., Van der Merwe, L., Joloba, M. L., Nanteza, A., ... & Kinyanda, E. (2017). Association between serotonin transporter gene polymorphisms and increased suicidal risk among HIV positive patients in Uganda. *BMC Genetics*, 18(1), 71.

Kalungi, A., Womersley, J. S., Kinyanda, E., Joloba, M. L., Ssembajjwe, W., Nsubuga, R. N., ... & Hemmings, S. (2019). Internalizing mental disorders and accelerated cellular aging among HIV+ children and adolescents in Uganda. *Frontiers in Genetics*, 10, 705.

Kamau, J. W., Kuria, W., Mathai, M., Atwoli, L., & Kangethe, R. (2012). Psychiatric morbidity among HIV-infected children and adolescents in a resource-poor Kenyan urban community. *AIDS Care*, 24(7), 836-842.

Kananen, L., Surakka, I., Pirkola, S., Suvisaari, J., Lönnqvist, J., Peltonen, L., ... & Hovatta, I. (2010). Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *PloS one*, 5(5), e10826.

Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Archives of General Psychiatry*, 68(5), 444-454.

Katrinli, S., Lori, A., Kilaru, V., Carter, S., Powers, A., Gillespie, C. F., ... & Smith, A. K. (2019). Association of HLA locus alleles with posttraumatic stress disorder. *Brain, Behavior, and Immunity*, 81, 655-658.

Kendler, K. S., Bulik, C. M., Silberg, J., Hettema, J. M., Myers, J., & Prescott, C. A. (2000). Childhood sexual abuse and adult psychiatric and substance use disorders in women: An epidemiological and cotwin control analysis. *Archives of General Psychiatry*, 57(10), 953-959.

Kendler, K. S., Karkowski, L. M., & Prescott, C. A. (1999). Causal relationship between stressful life events and the onset of major depression. *American Journal of Psychiatry*, 156(6), 837-841.

Stellenbosch University https://scholar.sun.ac.za

Kennedy, A. P., Binder, E. B., Bowman, D., Harenski, K., Ely, T., Cisler, J. M., ... & Kilts, C. D. (2012). A common TPH2 haplotype regulates the neural processing of a cognitive control demand. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 159(7), 829-840.

Kessler, R. C., & Frank, R. G. (1997). The impact of psychiatric disorders on work loss days. *Psychological Medicine*, 27(4), 861-873.

Kessler, R. C., & Wang, P. S. (2008). The descriptive epidemiology of commonly occurring mental disorders in the United States. *Annual Review of Public Health*, 29, 115-129.

Kiecolt-Glaser, J. K., Derry, H. M., & Fagundes, C. P. (2015). Inflammation: Depression fans the flames and feasts on the heat. *American Journal of Psychiatry*, 172(11), 1075-1091.

Kim, M. H., Mazenga, A. C., Devandra, A., Ahmed, S., Kazembe, P. N., Yu, X., ... & Sharp, C. (2014). Prevalence of depression and validation of the Beck Depression Inventory-II and the Children's Depression Inventory-Short amongst HIV-positive adolescents in Malawi. *Journal of the International AIDS Society*, 17(1), 18965.

Kinser, P. A., & Lyon, D. E. (2013). Major depressive disorder and measures of cellular aging: An integrative review. *Nursing Research and Practice*, 2013, 469070.

Kinyanda, E., Hoskins, S., Nakku, J., Nawaz, S., & Patel, V. (2011b). Prevalence and risk factors of major depressive disorder in HIV/AIDS as seen in semi-urban Entebbe district, Uganda. *BMC Psychiatry*, 11(1), 205.

Kinyanda, E., Kizza, R., Abbo, C., Ndyanabangi, S., & Levin, J. (2013). Prevalence and risk factors of depression in childhood and adolescence as seen in 4 districts of north-eastern Uganda. *BMC International Health and Human Rights*, 13(1), 19.

Kinyanda, E., Levin, J., Nakasujja, N., Birabwa, H., Nakku, J., Mpango, R., ... & Patel, V. (2018). Major depressive disorder: Longitudinal analysis of impact on clinical and behavioral outcomes in Uganda. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 78(2), 136-143.

Kinyanda, E., Nakasujja, N., Levin, J., Birabwa, H., Mpango, R., Grosskurth, H., ... & Patel, V. (2017). Major depressive disorder and suicidality in early HIV infection and its association with risk factors and negative outcomes as seen in semi-urban and rural Uganda. *Journal of Affective Disorders*, 212, 117-127.

Stellenbosch University https://scholar.sun.ac.za

Kinyanda, E., Salisbury, T. T., Levin, J., Nakasujja, N., Mpango, R. S., Abbo, C., ... & Patel, V. (2019). Rates, types and co-occurrence of emotional and behavioural disorders among perinatally HIV-infected youth in Uganda: the CHAKA study. *Social Psychiatry and Psychiatric Epidemiology*, 54(4), 415-425.

Kinyanda, E., Waswa, L., Baisley, K., & Maher, D. (2011a). Prevalence of severe mental distress and its correlates in a population-based study in rural south-west Uganda. *BMC Psychiatry*, 11(1), 97.

Kirkwood, B. R., & Sterne, J. A. C. (2003). Medical statistics. 2nd edn Oxford. UK: *Blackwell Publishing*. Page 420.

Kleinjan, D. A., & van Heyningen, V. (2005). Long-range control of gene expression: Emerging mechanisms and disruption in disease. *The American Journal of Human Genetics*, 76(1), 8-32.

Klumpers, F., Kroes, M. C., Heitland, I., Everaerd, D., Akkermans, S. E., Oosting, R. S., ... & Baas, J. M. (2015). Dorsomedial prefrontal cortex mediates the impact of serotonin transporter linked polymorphic region genotype on anticipatory threat reactions. *Biological Psychiatry*, 78(8), 582-589.

Klumpp, H., Fitzgerald, D. A., Cook, E., Shankman, S. A., Angstadt, M., & Phan, K. L. (2014). Serotonin transporter gene alters insula activity to threat in social anxiety disorder. *Neuroreport*, 25(12), 926.

Knizek, B. L., Mugisha, J., Osafo, J., & Kinyanda, E. (2017). Growing up HIV-positive in Uganda:“psychological immunodeficiency”? A qualitative study. *BMC Psychology*, 5(1), 30.

Ko, E., Seo, H. W., Jung, E. S., Kim, B. H., & Jung, G. (2016). The TERT promoter SNP rs2853669 decreases E2F1 transcription factor binding and increases mortality and recurrence risks in liver cancer. *Oncotarget*, 7(1), 684.

Koch, A. S., Forgas, J. P., & Matovic, D. (2013). Can negative mood improve your conversation? Affective influences on conforming to Grice's communication norms. *European Journal of Social Psychology*, 43(5), 326-334.

Koks, G., Prans, E., Tran, H. D. T., Ngo, N. B. T., Hoang, L. N. N., Tran, H. M. T., ... & Lättekivi, F. (2018). Genetic interaction between two VNTRs in the SLC6A4 gene regulates nicotine dependence in Vietnamese men. *Frontiers in pharmacology*, 9, 1398.

Kop, W. J., Stein, P. K., Tracy, R. P., Barzilay, J. I., Schulz, R., & Gottdiener, J. S. (2010). Autonomic nervous system dysfunction and inflammation contribute to the increased

Stellenbosch University https://scholar.sun.ac.za

cardiovascular mortality risk associated with depression. *Psychosomatic Medicine*, 72(7), 626.

Kristensen, A. S., Andersen, J., Jørgensen, T. N., Sørensen, L., Eriksen, J., Loland, C. J., ... & Gether, U. (2011). SLC6 neurotransmitter transporters: Structure, function, and regulation. *Pharmacological Reviews*, 63(3), 585-640.

Kurz, D. J., Decary, S., Hong, Y., Trivier, E., Akhmedov, A., & Erusalimsky, J. D. (2004). Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *Journal of Cell Science*, 117(11), 2417-2426.

Laas, K., Kiive, E., Mäestu, J., Vaht, M., Veidebaum, T., & Harro, J. (2017). Nice guys: Homozygocity for the TPH2-703G/T (rs4570625) minor allele promotes low aggressiveness and low anxiety. *Journal of Affective Disorders*, 215, 230-236.

Lachin, J. M. (1992). Power and sample size evaluation for the McNemar test with application to matched case-control studies. *Statistics in Medicine*, 11(9), 1239-1251.

Lan, T., Jia, X., Lin, D., & Liu, X. (2019). Stressful life events, depression, and non-suicidal self-injury among Chinese left-behind children: The moderating effects of self-esteem. *Frontiers in Psychiatry*, 10, 244.

Latsko, M. S., Gilman, T. L., Matt, L. M., Nylocks, K. M., Coifman, K. G., & Jasnow, A. M. (2016). A novel interaction between Tryptophan Hydroxylase 2 (TPH2) gene polymorphism (rs4570625) and BDNF Val66Met predicts a high-risk emotional phenotype in healthy subjects. *PloS one*, 11(10), e0162585.

Le Doare, K., Bland, R., & Newell, M. L. (2012). Neurodevelopment in children born to HIV-infected mothers by infection and treatment status. *Pediatrics*, 130(5), e1326-e1344.

Lee, H. J., Lee, M. S., Kang, R. H., Kim, H., Kim, S. D., Kee, B. S., ... & Oh, K. S. (2005). Influence of the serotonin transporter promoter gene polymorphism on susceptibility to posttraumatic stress disorder. *Depression and Anxiety*, 21(3), 135-139.

Lee, M. S., & Blackburn, E. H. (1993). Sequence-specific DNA primer effects on telomerase polymerization activity. *Molecular and Cellular Biology*, 13(10), 6586-6599.

Lee, S. S., Bohrson, C., Pike, A. M., Wheelan, S. J., & Greider, C. W. (2015). ATM kinase is required for telomere elongation in mouse and human cells. *Cell Reports*, 13(8), 1623–1632.

Leeansyah, E., Cameron, P. U., Solomon, A., Tennakoon, S., Velayudham, P., Gouillou, M., ... & Pierce, A. B. (2013). Inhibition of telomerase activity by human immunodeficiency virus (HIV) nucleos (t) ide reverse transcriptase inhibitors: a

Stellenbosch University https://scholar.sun.ac.za

potential factor contributing to HIV-associated accelerated aging. *The Journal of Infectious Diseases*, 207(7), 1157-1165.

Leonard, B. E. (2007). Inflammation, depression and dementia: are they connected? Neurochemical Research, 32(10), 1749-1756.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., ... & Murphy, D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274(5292), 1527-1531.

Levinson, D. F., Mostafavi, S., Milaneschi, Y., Rivera, M., Ripke, S., Wray, N. R., & Sullivan, P. F. (2014). Genetic studies of major depressive disorder: Why are there no genome-wide association study findings and what can we do about it? *Biological Psychiatry*, 76(7), 510-512.

Li, G., Wang, L., Cao, C., Fang, R., Hall, B. J., Elhai, J. D., & Liberzon, I. (2019). Post-traumatic stress symptoms of children and adolescents exposed to the 2008 Wenchuan Earthquake: A longitudinal study of 5-HTTLPR genotype main effects and gene–environment interactions. *International Journal of Psychology*. doi: 10.1002/ijop.12614.

Lin, J., Epel, E., & Blackburn, E. (2012). Telomeres and lifestyle factors: Roles in cellular aging. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* , 730(1-2), 85-89.

Lin, J., Sun, J., Wang, S., Milush, J. M., Baker, C. A., Coccia, M., ... & Epel, E. (2018). In vitro proinflammatory gene expression predicts in vivo telomere shortening: A preliminary study. *Psychoneuroendocrinology*, 96, 179-187.

Lin, P. Y., Huang, Y. C., & Hung, C. F. (2016). Shortened telomere length in patients with depression: A meta-analytic study. *Journal of Psychiatric Research*, 76, 84-93.

Lindert, J., von Ehrenstein, O. S., Grashow, R., Gal, G., Braehler, E., & Weisskopf, M. G. (2014). Sexual and physical abuse in childhood is associated with depression and anxiety over the life course: Systematic review and meta-analysis. *International Journal of Public Health*, 59(2), 359-372.

Lindqvist, D., Epel, E. S., Mellon, S. H., Penninx, B. W., Révész, D., Verhoeven, J. E., ... & Rosser, R. (2015). Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. *Neuroscience & Biobehavioral Reviews*, 55, 333-364.

Stellenbosch University https://scholar.sun.ac.za

Lindqvist, D., Simon, N. M., & Wolkowitz, O. M. (2019). Is Depression Associated With Accelerated Aging? Mechanisms and Implications. *In Neurobiology of Depression* (pp. 207-229). Academic Press.

Lipsky, R. H., Hu, X. Z., & Goldman, D. (2009). Additional functional variation at the SLC6A4 gene. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, 150(1), 153.

Liu, L., Wang, L., Cao, C., Cao, X., Zhu, Y., Liu, P., ... & Zhang, J. (2018). Serotonin transporter 5-HTTLPR genotype is associated with intrusion and avoidance symptoms of DSM-5 posttraumatic stress disorder (PTSD) in Chinese earthquake survivors. *Anxiety, Stress, & Coping*, 31(3), 318-327.

Lotfaliany, M., Agustini, B., Kowal, P., Berk, M., & Mohebbi, M. (2019). Co-occurrence of depression with chronic diseases among the older population living in low-and middle-income countries: A compound health challenge. *Annals of Clinical Psychiatry*, 31(2), 95-105.

Lung, F. W., Chen, N. C., & Shu, B. C. (2007). Genetic pathway of major depressive disorder in shortening telomeric length. *Psychiatric Genetics*, 17(3), 195.

Lwidiko, A., Kibusi, S. M., Nyundo, A., & Mpondo, B. C. (2018). Association between HIV status and depressive symptoms among children and adolescents in the Southern Highlands Zone, Tanzania: A case-control study. *PloS one*, 13(2), e0193145.

Malan, S., Hemmings, S., Kidd, M., Martin, L., & Seedat, S. (2011). Investigation of telomere length and psychological stress in rape victims. *Depression and Anxiety*, 28(12), 1081-1085.

Malee, K., Williams, P., Montepiedra, G., McCabe, M., Nichols, S., Sirois, P. A., ... & Kammerer, and the PACTG 219C Team, B. (2011). Medication adherence in children and adolescents with HIV infection: associations with behavioral impairment. *AIDS Patient Care and STDs*, 25(3), 191-200.

Malison, R. T., Price, L. H., Berman, R., Van Dyck, C. H., Pelton, G. H., Carpenter, L., ... & Baldwin, R. M. (1998). Reduced brain serotonin transporter availability in major depression as measured by [123I]-2β-carbomethoxy-3β-(4-iodophenyl) tropane and single photon emission computed tomography. *Biological Psychiatry*, 44(11), 1090-1098.

Malouff, J. M., & Schutte, N. S. (2017). Can psychological interventions increase optimism? A meta-analysis. *The Journal of Positive Psychology*, 12(6), 594-604.

Stellenbosch University https://scholar.sun.ac.za

Martin, J., Cleak, J., Willis-Owen, S. A. G., Flint, J., & Shifman, S. (2007). Mapping regulatory variants for the serotonin transporter gene based on allelic expression imbalance. *Molecular Psychiatry*, 12(5), 421.

Mason, D. R., Beck, P. L., & Muruve, D. A. (2012). Nucleotide-binding oligomerization domain-like receptors and inflammasomes in the pathogenesis of non-microbial inflammation and diseases. *Journal of Innate Immunity*, 4(1), 16-30.

Mavhu, W., Berwick, J., Chirawu, P., Makamba, M., Copas, A., Dirawo, J., ... & Mungofa, S. (2013). Enhancing psychosocial support for HIV positive adolescents in Harare, Zimbabwe. *PloS one*, 8(7), e70254.

Mayer, S. E., Prather, A. A., Puterman, E., Lin, J., Arenander, J., Coccia, M., ... & Epel, E. S. (2019). Cumulative lifetime stress exposure and leukocyte telomere length attrition: The unique role of stressor duration and exposure timing. *Psychoneuroendocrinology*, 104, 210-218.

McEwen, B. S. (2003). Mood disorders and allostatic load. *Biological Psychiatry*, 54(3), 200-207.

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiological Reviews*, 87(3), 873-904.

McKay, J. D., Hung, R. J., Gaborieau, V., Boffetta, P., Chabrier, A., Byrnes, G., ... & Rudnai, P. (2008). Lung cancer susceptibility locus at 5p15. 33. *Nature Genetics*, 40(12), 1404.

McLaughlin, K. A., Koenen, K. C., Hill, E. D., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2013). Trauma exposure and posttraumatic stress disorder in a national sample of adolescents. *Journal of the American Academy of Child & Adolescent Psychiatry*, 52(8), 815-830.

Meier, S. M., Trontti, K., Purves, K. L., Als, T. D., Grove, J., Laine, M., ... & Mortensen, P. B. (2019). Genetic variants associated with anxiety and stress-related disorders: A genome-wide association study and mouse-model study. *JAMA Psychiatry*, doi: 10.1001/jamapsychiatry.2019.1119.

Melin, B. S., Nordfjäll, K., Andersson, U., & Roos, G. (2012). h TERT cancer risk genotypes are associated with telomere length. *Genetic Epidemiology*, 36(4), 368-372.

Mellins, C. A., & Malee, K. M. (2013). Understanding the mental health of youth living with perinatal HIV infection: Lessons learned and current challenges. *Journal of the International AIDS Society*, 16(1), 18593.

Stellenbosch University https://scholar.sun.ac.za

Mellins, C. A., Elkington, K. S., Leu, C. S., Santamaria, E. K., Dolezal, C., Wiznia, A., ... & Abrams, E. J. (2012). Prevalence and change in psychiatric disorders among perinatally HIV-infected and HIV-exposed youth. *AIDS Care*, 24(8), 953-962.

Mendlowicz, M. V., & Stein, M. B. (2000). Quality of life in individuals with anxiety disorders. *American Journal of Psychiatry*, 157(5), 669-682.

Michalek, J. E., Kepa, A., Vincent, J., Frissa, S., Goodwin, L., Hotopf, M., ... & Powell, T. R. (2017). Genetic predisposition to advanced biological ageing increases risk for childhood-onset recurrent major depressive disorder in a large UK sample. *Journal of Affective Disorders*, 213, 207-213.

Michopoulos, V., Powers, A., Gillespie, C. F., Ressler, K. J., & Jovanovic, T. (2017). Inflammation in fear-and anxiety-based disorders: PTSD, GAD, and beyond. *Neuropsychopharmacology*, 42(1), 254.

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological Psychiatry*, 65(9), 732-741.

Miller, M. W., & Sadeh, N. (2014). Traumatic stress, oxidative stress and post-traumatic stress disorder: Neurodegeneration and the accelerated-aging hypothesis. *Molecular Psychiatry*, 19(11), 1156.

Miller, M. W., Lin, A. P., Wolf, E. J., & Miller, D. R. (2018). Oxidative Stress, inflammation, and neuroprogression in chronic PTSD. *Harvard Review of Psychiatry*, 26(2), 57-69.

Miloyan, B., Bienvenu, O. J., Brilot, B., & Eaton, W. W. (2018). Adverse life events and the onset of anxiety disorders. *Psychiatry Research*, 259, 488-492.

Mitchell, C., Hobcraft, J., McLanahan, S. S., Siegel, S. R., Berg, A., Brooks-Gunn, J., ... & Notterman, D. (2014). Social disadvantage, genetic sensitivity, and children's telomere length. *Proceedings of the National Academy of Sciences*, 111(16), 5944-5949.

Monroe, S. M., & Simons, A. D. (1991). Diathesis-stress theories in the context of life stress research: implications for the depressive disorders. *Psychological Bulletin*, 110(3), 406.

Montejano, R., Stella-Ascariz, N., Monge, S., Bernardino, J. I., Pérez-Valero, I., Montes, M. L., ... & Arnalich, F. (2017). Impact of antiretroviral treatment containing tenofovir difumarate on the telomere length of aviremic HIV-infected patients. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 76(1), 102-109.

Montejano, R., Stella-Ascariz, N., Monge, S., Bernardino, J. I., Pérez-Valero, I., Montes, M. L., ... & Rodriguez-Centeno, J. (2018). Impact of nucleos(t)ide reverse transcriptase

Stellenbosch University https://scholar.sun.ac.za

inhibitors on blood telomere length changes in a prospective cohort of aviremic HIV–infected adults. *The Journal of Infectious Diseases*, 218(10), 1531-1540.

Moreno-Villanueva, M., Morath, J., Vanhooren, V., Elbert, T., Kolassa, S., Libert, C., ... & Kolassa, I. T. (2013). N-glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder. *Translational Psychiatry*, 3(10), e320.

Morris, A., Gabert-Quillen, C., & Delahanty, D. (2012). The association between parent PTSD/depression symptoms and child PTSD symptoms: A meta-analysis. *Journal of Pediatric Psychology*, 37(10), 1076-1088.

Mpango, R. S., Kinyanda, E., Rukundo, G. Z., Gadow, K. D., & Patel, V. (2017). Cross-cultural adaptation of the child and adolescent symptom Inventory-5 (CASI-5) for use in central and south-western Uganda: the CHAKA project. *Tropical Doctor*, 47(4), 347-354.

Müezzinler, A., Zaineddin, A. K., & Brenner, H. (2013). A systematic review of leukocyte telomere length and age in adults. *Ageing Research Reviews*, 12(2), 509-519.

Munafò, M. R., Brown, S. M., & Hariri, A. R. (2008). Serotonin transporter (5-HTTLPR) genotype and amygdala activation: A meta-analysis. *Biological Psychiatry*, 63(9), 852-857.

Munafò, M. R., Durrant, C., Lewis, G., & Flint, J. (2009a). Gene × environment interactions at the serotonin transporter locus. *Biological Psychiatry*, 65(3), 211-219.

Munafò, M. R., Freimer, N. B., Ng, W., Ophoff, R., Veijola, J., Miettunen, J., ... & Flint, J. (2009b). 5-HTTLPR genotype and anxiety related personality traits: A meta-analysis and new data. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 150(2), 271-281.

Munkholm, K., Paludan-Müller, A. S., & Boesen, K. (2019). Considering the methodological limitations in the evidence base of antidepressants for depression: a reanalysis of a network meta-analysis. *British Medical Journal open*, 9(6), e024886.

Mupambireyi, Z., Bernays, S., Bwakura-Dangarembizi, M., & Cowan, F. M. (2014). “I don't feel shy because I will be among others who are just like me…”: The role of support groups for children perinatally infected with HIV in Zimbabwe. *Children and Youth Services Review*, 45, 106-113.

Murray, C. J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., ... & Aboyans, V. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, 380(9859), 2197-2223.

Stellenbosch University https://scholar.sun.ac.za

Musci, R. J., Augustinavicius, J. L., & Volk, H. (2019). Gene-environment interactions in psychiatry: Recent evidence and clinical implications. *Current Psychiatry Reports*, 21(9), 81.

Mushiroda, T., Wattanapokayakit, S., Takahashi, A., Nukiwa, T., Kudoh, S., Ogura, T., ... & Pirfenidone Clinical Study Group. (2008). A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. *Journal of Medical Genetics*, 45(10), 654-656.

Mushtaq, D., Ali, A., Margoob, M. A., Murtaza, I., & Andrade, C. (2012). Association between serotonin transporter gene promoter-region polymorphism and 4-and 12-week treatment response to sertraline in posttraumatic stress disorder. *Journal of Affective Disorders*, 136(3), 955-962.

Musinguzi, K., Obuku, A., Nakasujja, N., Birabwa, H., Nakku, J., Levin, J., & Kinyanda, E. (2018). Association between major depressive disorder and pro-inflammatory cytokines and acute phase proteins among HIV-1 positive patients in Uganda. *BMC Immunology*, 19(1), 1.

Musisi, S., & Kinyanda, E. (2003, April). Psychological problems of HIV sero-positive adolescents as seen at Mildmay Center, Kampala. In *Proceeds of The Eastern African Regional Psychiatric Annual Conference*, Nairobi, Kenya.

Musisi, S., & Kinyanda, E. (2009). Emotional and behavioural disorders in HIV seropositive adolescents in urban Uganda. *East African Medical Journal*, 86(1).

Musisi, S., Kinyanda, E., Nakasujja, N., & Nakigudde, J. (2007). A comparison of the behavioral and emotional disorders of primary school-going orphans and non-orphans in Uganda. *African Health Sciences*, 7(4).

Mutumba, M., Bauermeister, J. A., Harper, G. W., Musiime, V., Lepkowski, J., Resnicow, K., & Snow, R. C. (2017). Psychological distress among Ugandan adolescents living with HIV: Examining stressors and the buffering role of general and religious coping strategies. *Global Public Health*, 12(12), 1479-1491.

Nachman, S., Chernoff, M., Williams, P., Hodge, J., Heston, J., & Gadow, K. D. (2012). Human immunodeficiency virus disease severity, psychiatric symptoms, and functional outcomes in perinatally infected youth. *Archives of Pediatrics & Adolescent Medicine*, 166(6), 528-535.

Stellenbosch University https://scholar.sun.ac.za

National Center for Biotechnology Information. (2019). STIN2-VNTR serotonin transporter intronic VNTR enhancer [Homo sapiens (human)]. Retrieved from https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=110806307.

National Center for Biotechnology Information. (2019). ZFC3H1 zinc finger C3H1-type containing [Homo sapiens (human)] Retrieved from: https://www.ncbi.nlm.nih.gov/gene/196441.

National Institute of Mental Health. Depression nimh.nih.gov. https://www.nimh.nih.gov/health/topics/depression/index.shtml. Accessed 24 July 2019.

Needham, B. L., Fernandez, J. R., Lin, J., Epel, E. S., & Blackburn, E. H. (2012). Socioeconomic status and cell aging in children. *Social Science & Medicine*, 74(12), 1948-1951.

Needham, B. L., Mezuk, B., Bareis, N., Lin, J., Blackburn, E. H., & Epel, E. S. (2015). Depression, anxiety and telomere length in young adults: evidence from the National Health and Nutrition Examination Survey. *Molecular Psychiatry*, 20(4), 520.

Ng, F., Berk, M., Dean, O., & Bush, A. I. (2008). Oxidative stress in psychiatric disorders: Evidence base and therapeutic implications. *International Journal of Neuropsychopharmacology*, 11(6), 851-876.

Nievergelt, C. M., Maihofer, A. X., Klengel, T., Atkinson, E. G., Chen, C. Y., Choi, K. W., ... & Levey, D. F. (2019). International meta-analysis of PTSD genome-wide association studies identifies sex-and ancestry-specific genetic risk loci. *Nature Communications*, 10(1), 1-16.

Nightingale, V. R., Sher, T. G., Mattson, M., Thilges, S., & Hansen, N. B. (2011). The effects of traumatic stressors and HIV-related trauma symptoms on health and health related quality of life. *AIDS and Behavior*, 15(8), 1870–1878.

Njajou, O. T., Cawthon, R. M., Damcott, C. M., Wu, S. H., Ott, S., Garant, M. J., ... & Hsueh, W. C. (2007). Telomere length is paternally inherited and is associated with parental lifespan. *Proceedings of the National Academy of Sciences*, 104(29), 12135-12139.

Notterman, D. A., & Mitchell, C. (2015). Epigenetics and understanding the impact of social determinants of health. *Pediatric Clinics*, 62(5), 1227-1240.

O'Connor, R. C., Rasmussen, S., & Hawton, K. (2010). Predicting depression, anxiety and self-harm in adolescents: The role of perfectionism and acute life stress. *Behaviour Research and Therapy*, 48(1), 52-59.

Stellenbosch University https://scholar.sun.ac.za

O'Donovan, A., Epel, E., Lin, J., Wolkowitz, O., Cohen, B., Maguen, S., ... & Neylan, T. C. (2011). Childhood trauma associated with short leukocyte telomere length in posttraumatic stress disorder. *Biological Psychiatry*, 70(5), 465-471.

Oeseburg, H., de Boer, R. A., van Gilst, W. H., & van der Harst, P. (2010). Telomere biology in healthy aging and disease. *Pflügers Archiv-European Journal of Physiology*, 459(2), 259-268.

O'Gara, C., Knight, J., Stapleton, J., Luty, J., Neale, B., Nash, M., ... & Collier, D. (2008). Association of the serotonin transporter gene, neuroticism and smoking behaviours. *Journal of Human Genetics*, 53(3), 239.

Ogilvie, A. D., Battersby, S., Fink, G., Harmar, A. J., Goodwin, G. M., Bubb, V. J., & Smith, C. D. (1996). Polymorphism in serotonin transporter gene associated with susceptibility to major depression. *The Lancet*, 347(9003), 731-733.

Okereke, O. I., Prescott, J., Wong, J. Y., Han, J., Rexrode, K. M., & De Vivo, I. (2012). High phobic anxiety is related to lower leukocyte telomere length in women. *PloS one*, 7(7), e40516.

Olivier, B. (2015). Serotonin: A never-ending story. *European Journal of Pharmacology*, 753, 2-18.

Ollendick, T. H., & King, N. J. (1994). Diagnosis, assessment, and treatment of internalizing problems in children: The role of longitudinal data. *Journal of Consulting and Clinical Psychology*, 62(5), 918.

Ortin, A., Elkington, K. S., Eisenberg, R., Miranda, R., Canino, G., Bird, H. R., & Duarte, C. S. (2019). Suicide attempts and course of suicidal ideation among Puerto Rican early adolescents. *Journal of Abnormal Child Psychology*, 1-12.

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., ... & Schatzberg, A. F. (2016). Major depressive disorder. *Nature Reviews Disease Primers*, 2, 16065.

Ottenhof, K. W., Sild, M., Levesque, M. L., Ruhe, H. G., & Booij, L. (2018). TPH2 polymorphisms across the spectrum of psychiatric morbidity: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews*, 92, 29-42.

Parks, C. G., Miller, D. B., McCanlies, E. C., Cawthon, R. M., Andrew, M. E., DeRoo, L. A., & Sandler, D. P. (2009). Telomere length, current perceived stress, and urinary stress hormones in women. *Cancer Epidemiology and Prevention Biomarkers*, 18(2), 551-560.

Stellenbosch University https://scholar.sun.ac.za

Pathai, S., Lawn, S. D., Gilbert, C. E., McGuinness, D., McGlynn, L., Weiss, H. A., ... & Bekker, L. G. (2013). Accelerated biological ageing in HIV-infected individuals in South Africa: A case–control study. *AIDS (London, England)*, 27(15), 2375.

Pergamin-Hight, L., Bakermans-Kranenburg, M. J., van IJzendoorn, M. H., & Bar-Haim, Y. (2012). Variations in the promoter region of the serotonin transporter gene and biased attention for emotional information: a meta-analysis. *Biological Psychiatry*, 71(4), 373-379.

Peterson, R. E., Kuchenbaecker, K., Walters, R. K., Chen, C. Y., Popejoy, A. B., Periyasamy, S., ... & Carey, C. E. (2019). Genome-wide association studies in ancestrally diverse populations: Opportunities, methods, pitfalls, and recommendations. *Cell*, 179(3), 589-603.

Philibert, R. A., Sandhu, H., Hollenbeck, N., Gunter, T., Adams, W., & Madan, A. (2008). The relationship of 5HTT (SLC6A4) methylation and genotype on mRNA expression and liability to major depression and alcohol dependence in subjects from the Iowa Adoption Studies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 147(5), 543-549.

Pilowsky, D. J., Olfson, M., Gameroff, M. J., Wickramaratne, P., Blanco, C., Feder, A., ... & Weissman, M. M. (2006). Panic disorder and suicidal ideation in primary care. *Depression and Anxiety*, 23(1), 11-16.

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry*, 55(1), 56-64.

Plomin, R., & Davis, O. S. (2009). The future of genetics in psychology and psychiatry: Microarrays, genome-wide association, and non-coding RNA. *Journal of Child Psychology and Psychiatry*, 50(1-2), 63-71.

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry*, 56(3), 345-365.

Popejoy, A. B., & Fullerton, S. M. (2016). Genomics is failing on diversity. *Nature News*, 538(7624), 161.

Popoli, M., Yan, Z., McEwen, B. S., & Sanacora, G. (2012). The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews Neuroscience*, 13(1), 22.

Stellenbosch University https://scholar.sun.ac.za

Porcelli, S., Fabbri, C., Drago, A., Gibiino, S., De Ronchi, D., & Serretti, A. (2011). Genetics and antidepressants: Where we are. *Clin Neuropsychiatry*, 8(2), 99-150.

Pratt, L. A., Druss, B. G., Manderscheid, R. W., & Walker, E. R. (2016). Excess mortality due to depression and anxiety in the United States: Results from a nationally representative survey. *General Hospital Psychiatry*, 39, 39-45.

Pristerà, A., Lin, W., Kaufmann, A. K., Brimblecombe, K. R., Threlfell, S., Dodson, P. D., ... & Ang, S. L. (2015). Transcription factors FOXA1 and FOXA2 maintain dopaminergic neuronal properties and control feeding behavior in adult mice. *Proceedings of the National Academy of Sciences*, 112(35), E4929-E4938.

Radcliffe, J., Fleisher, C. L., Hawkins, L. A., Tanney, M., Kassam-Adams, N., Ambrose, C., & Rudy, B. J. (2007). Posttraumatic stress and trauma history in adolescents and young adults with HIV. *AIDS Patient Care and STDs*, 21(7), 501-508.

Ramunas, J., Yakubov, E., Brady, J. J., Corbel, S. Y., Holbrook, C., Brandt, M., ... & Blau, H. M. (2015). Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells. *The FASEB Journal*, 29(5), 1930-1939.

Rapee RM (2018). Anxiety disorders in children and adolescents: Nature, development, treatment and prevention. In Rey JM (ed), *IACAPAP e-Textbook of Child and Adolescent Mental Health*. Geneva: International Association for Child and Adolescent Psychiatry and Allied Professions.

Rayner, C., Coleman, J. R., Purves, K. L., Hodsoll, J., Goldsmith, K., Alpers, G. W., ... & Creswell, C. (2019). A genome-wide association meta-analysis of prognostic outcomes following cognitive behavioural therapy in individuals with anxiety and depressive disorders. *Translational Psychiatry*, 9(1), 150.

Regier, D. A., Kuhl, E. A., & Kupfer, D. J. (2013). The DSM-5: Classification and criteria changes. *World Psychiatry*, 12(2), 92-98.

Rehkopf, D. H., Needham, B. L., Lin, J., Blackburn, E. H., Zota, A. R., Wojcicki, J. M., & Epel, E. S. (2016). Leukocyte telomere length in relation to 17 biomarkers of cardiovascular disease risk: a cross-sectional study of US adults. *PLoS Medicine*, 13(11), e1002188.

Reinherz, H. Z., Paradis, A. D., Giaconia, R. M., Stashwick, C. K., & Fitzmaurice, G. (2003). Childhood and adolescent predictors of major depression in the transition to adulthood. *American Journal of Psychiatry*, 160(12), 2141-2147.

Stellenbosch University https://scholar.sun.ac.za

Remien, R. H., & Mellins, C. A. (2007). Long-term psychosocial challenges for people living with HIV: Let's not forget the individual in our global response to the pandemic. *AIDS*, 21(Suppl 5):S55-S63.

Reuter, M., Kuepper, Y., & Hennig, J. (2007). Association between a polymorphism in the promoter region of the TPH2 gene and the personality trait of harm avoidance. *International Journal of Neuropsychopharmacology*, 10(3), 401-404.

Revenson, T., Griva, K., Luszczynska, A., Morrison, V., Panagopoulou, E., Vilchinsky, N., & Hagedoorn, M. (2016). *Caregiving in the illness context*. London, UK: Palgrave Pivot.

Rey JM, Bella-Awusah TT, Jing L (2015). Depression in children and adolescents. In Rey JM (ed), *IACAPAP e-Textbook of Child and Adolescent Mental Health*. Geneva: International Association for Child and Adolescent Psychiatry and Allied Professions.

Reynolds, C. R., & Richmond, B. O. (1985). Revised children's manifest anxiety scale (RCMAS). Losa Angeles, CA: Western Psychological Services.

Ridout, S. J., Ridout, K. K., Kao, H. T., Carpenter, L. L., Philip, N. S., Tyrka, A. R., & Price, L. H. (2015). Telomeres, early-life stress and mental illness. *Advances in Psychosomatic Medicine*, 34, 92-108.

Ripke, S., Wray, N. R., Lewis, C. M., Hamilton, S. P., Weissman, M. M., Breen, G., ... & Heath, A. C. (2013). A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular Psychiatry*, 18(4), 497-511.

Roberts, A. L., Koenen, K. C., Chen, Q., Gilsanz, P., Mason, S. M., Prescott, J., ... & De, I. V. (2017). Posttraumatic stress disorder and accelerated aging: PTSD and leukocyte telomere length in a sample of civilian women. *Depression and Anxiety*, 34(5), 391-400.

Robles, T. F., Slatcher, R. B., Trombello, J. M., & McGinn, M. M. (2014). Marital quality and health: A meta-analytic review. *Psychological Bulletin*, 140(1), 140.

Rode, L., Nordestgaard, B. G., & Bojesen, S. E. (2016). Long telomeres and cancer risk among 95 568 individuals from the general population. *International Journal of Epidemiology*, 45(5), 1634-1643.

Rosliwati, M. Y., Rohayah, H., Jamil, B. Y. M., Zaharah, S., & Med, M. (2008). Validation of the Malay version of Children Depression Inventory (CDI) among children and adolescents attending outpatient Clinics in Kota Bharu, Kelantan. *Malaysian Journal of Psychiatry*, 17(1).

Stellenbosch University https://scholar.sun.ac.za

Roy-Byrne, P. P., Davidson, K. W., Kessler, R. C., Asmundson, G. J., Goodwin, R. D., Kubzansky, L., ... & Stein, M. B. (2008). Anxiety disorders and comorbid medical illness. *Focus*, 6(4), 467-485.

Rudnick, G. (2006). Serotonin transporters–structure and function. *The Journal of Membrane Biology*, 213(2), 101-110.

Šagud, M., Jakšić, N., Vuksan-Ćusa, B., Lončar, M., Lončar, I., Mihaljević Peleš, A., ... & Jakovljević, M. (2017). Cardiovascular disease risk factors in patients with posttraumatic stress disorder (ptsd): A narrative review. *Psychiatria Danubina*, 29(4), 421-430.

Sanders, J. L., & Newman, A. B. (2013). Telomere length in epidemiology: A biomarker of aging, age-related disease, both, or neither? *Epidemiologic Reviews*, 35(1), 112-131.

Sarandol, A., Sarandol, E., Eker, S. S., Erdinc, S., Vatansever, E., & Kirli, S. (2007). Major depressive disorder is accompanied with oxidative stress: Short-term antidepressant treatment does not alter oxidative–antioxidative systems. *Human Psychopharmacology: Clinical and Experimental*, 22(2), 67-73.

Saul, A., Taylor, B., Simpson Jr, S., Ponsonby, A. L., Blizzard, L., Dwyer, T., ... & van der Mei, I. A. (2019). Polymorphism in the serotonin transporter gene polymorphisms (5-HTTLPR) modifies the association between significant life events and depression in people with multiple sclerosis. *Multiple Sclerosis Journal*, 25(6), 848-855.

Schaakxs, R., Verhoeven, J. E., Voshaar, R. C. O., Comijs, H. C., & Penninx, B. W. (2015). Leukocyte telomere length and late-life depression. *The American Journal of Geriatric Psychiatry*, 23(4), 423-432.

Schiavone, S., Jaquet, V., Trabace, L., & Krause, K. H. (2013). Severe life stress and oxidative stress in the brain: From animal models to human pathology. *Antioxidants & Redox Signaling*, 18(12), 1475-1490.

Schosser, A., Butler, A. W., Uher, R., Ng, M. Y., Cohen-Woods, S., Craddock, N., ... & Hauser, J. (2013). Genome-wide association study of co-occurring anxiety in major depression. *The World Journal of Biological Psychiatry*, 14(8), 611-621.

Schutte, N. S., & Malouff, J. M. (2015). The association between depression and leukocyte telomere length: A meta-analysis. *Depression and Anxiety*, 32(4), 229-238.

Seripa, D., Iurescia, S., Rinaldi, M., Pilotto, A., Paroni, G., Bazzano, S., Antonelli, A., Gravina, C. et al. (2013). The serotonin transporter gene-linked polymorphic region: Identification and functional characterization of novel alleles. *European Geriatric Medicine*, 4(1):S100.

Stellenbosch University https://scholar.sun.ac.za

Serretti, A., Kato, M., De Ronchi, D., & Kinoshita, T. (2007). Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Molecular Psychiatry*, 12(3), 247.

Shafiee, M., Ahmadnezhad, M., Tayefi, M., Arekhi, S., Vatanparast, H., Esmaeili, H., ... & Ghayour-Mobarhan, M. (2018). Depression and anxiety symptoms are associated with prooxidant-antioxidant balance: A population-based study. *Journal of Affective Disorders*, 238, 491-498.

Shalev, I. (2012). Early life stress and telomere length: investigating the connection and possible mechanisms: A critical survey of the evidence base, research methodology and basic biology. *Bioessays*, 34(11), 943-952.

Shalev, I., Entringer, S., Wadhwa, P. D., Wolkowitz, O. M., Puterman, E., Lin, J., & Epel, E. S. (2013). Stress and telomere biology: A lifespan perspective. *Psychoneuroendocrinology*, 38(9), 1835-1842.

Shalev, I., Moffitt, T. E., Braithwaite, A. W., Danese, A., Fleming, N. I., Goldman-Mellor, S., ... & Robertson, S. P. (2014). Internalizing disorders and leukocyte telomere erosion: A prospective study of depression, generalised anxiety disorder and post-traumatic stress disorder. *Molecular Psychiatry*, 19(11), 1163-1170.

Sharma, S., Powers, A., Bradley, B., & Ressler, K. J. (2016). Gene× environment determinants of stress-and anxiety-related disorders. *Annual Review of Psychology*, 67, 239-261.

Sharpley, C. F., Palanisamy, S. K., Glyde, N. S., Dillingham, P. W., & Agnew, L. L. (2014). An update on the interaction between the serotonin transporter promoter variant (5-HTTLPR), stress and depression, plus an exploration of non-confirming findings. *Behavioural Brain Research*, 273, 89-105.

Shay, J. W. (2016). Role of telomeres and telomerase in aging and cancer. *Cancer Discovery*, 6(6), 584-593.

Sheerin, C. M., Lind, M. J., Brown, E. A., Gardner, C. O., Kendler, K. S., & Amstadter, A. B. (2018). The impact of resilience and subsequent stressful life events on MDD and GAD. *Depression and Anxiety*, 35(2), 140-147.

Shen, Q., Zhang, Z., Yu, L., Cao, L., Zhou, D., Kan, M., ... & Liu, Y. (2011). Common variants near TERC are associated with leukocyte telomere length in the Chinese Han population. *European Journal of Human Genetics*, 19(6), 721.

Sheng, J., Liu, S., Wang, Y., Cui, R., & Zhang, X. (2017). The link between depression and chronic pain: Neural mechanisms in the brain. *Neural Plasticity*, 2017, 9724371.

Stellenbosch University https://scholar.sun.ac.za

Shiau, S., Strehlau, R., Shen, J., Violari, A., Patel, F., Liberty, A., ... & Coovadia, A. (2018). Biomarkers of aging in HIV-infected children on suppressive antiretroviral therapy. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 78(5), 549-556.

Shiels, P. G., McGlynn, L. M., MacIntyre, A., Johnson, P. C., Batty, G. D., Burns, H., ... & McGinty, A. (2011). Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *PloS one*, 6(7), e22521.

Shin, D., Shin, J., & Lee, K. W. (2019). Effects of inflammation and depression on telomere length in young adults in the United States. *Journal of Clinical Medicine*, 8(5), 711.

Shishkina, G. T., Kalinina, T. S., & Dygalo, N. N. (2007). Up-regulation of tryptophan hydroxylase-2 mRNA in the rat brain by chronic fluoxetine treatment correlates with its antidepressant effect. *Neuroscience*, 150(2), 404-412.

Silberg, J. L., Rutter, M., & Eaves, L. (2001). Genetic and environmental influences on the temporal association between earlier anxiety and later depression in girls. *Biological Psychiatry*, 49(12), 1040-1049.

Silverman, W. K., Fleisig, W., Rabian, B., & Peterson, R. A. (1991). Childhood anxiety sensitivity index. *Journal of Clinical Child and Adolescent Psychology*, 20(2), 162-168.

Simon, N. M., Smoller, J. W., McNamara, K. L., Maser, R. S., Zalta, A. K., Pollack, M. H., ... & Wong, K. K. (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biological Psychiatry*, 60(5), 432-435.

Simon, N. M., Walton, Z. E., Bui, E., Prescott, J., Hoge, E., Keshaviah, A., ... & Mischoulon, D. (2015). Telomere length and telomerase in a well-characterized sample of individuals with major depressive disorder compared to controls. *Psychoneuroendocrinology*, 58, 9-22.

Simoni, J. M., Safren, S. A., Manhart, L. E., Lyda, K., Grossman, C. I., Rao, D., ... & DiClemente, R. (2011). Challenges in addressing depression in HIV research: Assessment, cultural context, and methods. *AIDS and Behavior*, 15(2), 376-388.

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: A social signal transduction theory of depression. *Psychological Bulletin*, 140(3), 774.

Smoller, J. W. (2016). The genetics of stress-related disorders: PTSD, depression, and anxiety disorders. *Neuropsychopharmacology*, 41(1), 297.

Solomon, A., Tennakoon, S., Leeansyah, E., Arribas, J., Hill, A., Van Delft, Y., ... & Lewin, S. R. (2014). No difference in the rate of change in telomere length or telomerase

Stellenbosch University https://scholar.sun.ac.za

activity in HIV-infected patients after three years of darunavir/ritonavir with and without nucleoside analogues in the MONET trial. *PLoS One*, 9(11), e109718.

Spiers, J. G., Chen, H. J. C., Sernia, C., & Lavidis, N. A. (2015). Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. *Frontiers in Neuroscience*, 8, 456.

Springer, S. A., Dushaj, A., & Azar, M. M. (2012). The impact of DSM-IV mental disorders on adherence to combination antiretroviral therapy among adult persons living with HIV/AIDS: A systematic review. *AIDS and Behavior*, 16(8), 2119-2143.

Srinivasa, S., Fitch, K. V., Petrow, E., Burdo, T. H., Williams, K. C., Lo, J., ... & Grinspoon, S. K. (2014). Soluble CD163 is associated with shortened telomere length in HIV-infected patients. *Journal of Acquired Immune Deficiency Syndromes* (1999), 67(4), 414-418.

Stein, M. B., Schork, N. J., & Gelernter, J. (2008). Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology*, 33(2), 312.

Stella-Ascariz, N., Montejano, R., Rodriguez-Centeno, J., Alejos, B., Schwimmer, C., Bernardino, J. I., ... & de Miguel, R. (2018). Blood telomere length changes after Ritonavir-boosted Darunavir combined with Raltegravir or Tenofovir-Emtricitabine in antiretroviral-naive adults infected with HIV-1. *The Journal of Infectious Diseases*, 218(10), 1523-1530.

Stenbäck, V., Jagalur Mutt, S., Leppäluoto, J., Gagnon, D., Mäkelä, K. A., Jokelainen, J., ... & Herzig, K. H. (2019). Effect of physical activity on telomere length in elderly adults-the Oulu Cohort 1945. *Frontiers in Physiology*, 10, 444.

Sullivan, P. F., Agrawal, A., Bulik, C. M., Andreassen, O. A., Børglum, A. D., Breen, G., ... & Mathews, C. A. (2017). Psychiatric genomics: An update and an agenda. *American Journal of Psychiatry*, 175(1), 15-27.

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: Review and meta-analysis. *American Journal of Psychiatry*, 157(10), 1552-1562.

Tandon, M., Si, X., & Luby, J. (2011). Preschool onset attention-deficit/hyperactivity disorder: Course and predictors of stability over 24 months. *Journal of Child and Adolescent Psychopharmacology*, 21(4), 321-330.

Stellenbosch University https://scholar.sun.ac.za

Taylor, J., Tyekucheva, S., King, D. C., Hardison, R. C., Miller, W., & Chiaromonte, F. (2006). ESPERR: Learning strong and weak signals in genomic sequence alignments to identify functional elements. *Genome Research*, 16(12), 1596-1604.

Teyssier, J. R., Chauvet-Gelinier, J. C., Ragot, S., & Bonin, B. (2012). Up-regulation of leucocytes genes implicated in telomere dysfunction and cellular senescence correlates with depression and anxiety severity scores. *PloS one*, 7(11), e49677.

Theall, K. P., Brett, Z. H., Shirtcliff, E. A., Dunn, E. C., & Drury, S. S. (2013). Neighborhood disorder and telomeres: Connecting children's exposure to community level stress and cellular response. *Social Science & Medicine*, 85, 50-58.

Tishkoff, S. A., Reed, F. A., Friedlaender, F. R., Ehret, C., Ranciaro, A., Froment, A., ... & Ibrahim, M. (2009). The genetic structure and history of Africans and African Americans. *Science*, 324(5930), 1035-1044.

Trickey, D., Siddaway, A. P., Meiser-Stedman, R., Serpell, L., & Field, A. P. (2012). A meta-analysis of risk factors for post-traumatic stress disorder in children and adolescents. *Clinical Psychology Review*, 32(2), 122-138.

Tsakiri, K. D., Cronkhite, J. T., Kuan, P. J., Xing, C., Raghu, G., Weissler, J. C., ... & Garcia, C. K. (2007). Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proceedings of the National Academy of Sciences*, 104(18), 7552-7557.

Turygin, N., Matson, J. L., Beighley, J., & Adams, H. (2013). The effect of DSM-5 criteria on the developmental quotient in toddlers diagnosed with autism spectrum disorder. *Developmental Neurorehabilitation*, 16(1), 38-43.

Tyrka, A. R., Parade, S. H., Price, L. H., Kao, H. T., Porton, B., Philip, N. S., ... & Carpenter, L. L. (2016). Alterations of mitochondrial DNA copy number and telomere length with early adversity and psychopathology. *Biological Psychiatry*, 79(2), 78-86.

UNAIDS. (2010). UNAIDS Report on the Global AIDS Epidemic.Retrived from from: https://www.unaids.org/globalreport/Global report.htm. Accessed June 16, 2019.

UNAIDS. (2018). HIV & AIDS statistics2018 fact sheet. Retrived from http://www.unaids.org/en/resources/fact-sheet. Accessed on October 27 2018.

UNAIDS. (2019). Global HIV & AIDS statistics - 2019 fact sheet. https://www.unaids.org/en/resources/fact-sheet. Accessed on August 10 2019.

UPHIA (2016–2017). Uganda population based HIV impact assessment 2017 . Retrived from:http://www.afro.who.int/sites/default/files/2017 08/UPHIA%20Uganda%20factsheet.pdf. Accessed 02 June 2019.

Stellenbosch University https://scholar.sun.ac.za

US National Library of Medicine. (2019). Solute carrier family 6 member 4. Retrieved from https://ghr.nlm.nih.gov/gene/SLC6A4#location.

Van Den Bogaert, A., Sleegers, K., De Zutter, S., Heyrman, L., Norrback, K. F., Adolfsson, R., ... & Del-Favero, J. (2006). Association of brain-specific tryptophan hydroxylase, TPH2, with unipolar and bipolar disorder in a Northern Swedish, isolated population. *Archives of General Psychiatry*, 63(10), 1103-1110.

Van den Buuse, M., & Hale, M. W. (2019). Serotonin in Stress. In *Stress: Physiology, Biochemistry, and Pathology* (pp. 115-123). Cambridge, MA: Academic Press.

Van der Merwe, C., Mwesiga, E. K., McGregor, N. W., Ejigu, A., Tilahun, A. W., Kalungi, A., ... & Majara, L. (2018). Advancing neuropsychiatric genetics training and collaboration in Africa. *The Lancet Global Health*, 6(3), e246-e247.

Van Rie, A., Harrington, P. R., Dow, A., & Robertson, K. (2007). Neurologic and neurodevelopmental manifestations of pediatric HIV/AIDS: A global perspective. *European Journal of Paediatric Neurology*, 11(1), 1-9.

Varni, S. E., Miller, C. T., McCuin, T., & Solomon, S. (2012). Disengagement and engagement coping with HIV/AIDS stigma and psychological well-being of people with HIV/AIDS. *Journal of Social and Clinical Psychology*, 31(2), 123-150.

Vasa-Nicotera, M., Brouilette, S., Mangino, M., Thompson, J. R., Braund, P., Clemitson, J. R., ... & Samani, N. J. (2005). Mapping of a major locus that determines telomere length in humans. *The American Journal of Human Genetics*, 76(1), 147-151.

Verhoeven, J. E., & Penninx, B. W. J. H. (2017). Associations between depression, anxiety and telomere length in a large Dutch psychiatric cohort study. *Tijdschrift Voor Psychiatrie*, 59(6), 350-359.

Verhoeven, J. E., Penninx, B. W., & Milaneschi, Y. (2019). Unraveling the association between depression and telomere length using genomics. *Psychoneuroendocrinology*, 102, 121-127.

Verhoeven, J. E., Révész, D., Epel, E. S., Lin, J., Wolkowitz, O. M., & Penninx, B. W. (2014). Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. *Molecular Psychiatry*, 19(8), 895.

Verhoeven, J. E., Revesz, D., van Oppen, P., Epel, E. S., Wolkowitz, O. M., & Penninx, B. W. (2015). Anxiety disorders and accelerated cellular ageing. *The British Journal of Psychiatry*, 206(5), 371-378.

Von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, 27(7), 339-344.

Stellenbosch University https://scholar.sun.ac.za

Wade, M., Fox, N. A., Zeanah, C. H., Nelson, C. A., & Drury, S. S. (2019). Telomere length and psychopathology: Specificity and direction of effects within the Bucharest Early Intervention Project. *Journal of the American Academy of Child & Adolescent Psychiatry*, pii: S0890-8567(19)30136-4. doi: 10.1016/j.jaac.2019.02.013.

Walkup, J., Akincigil, A., Bilder, S., Rosato, N. S., & Crystal, S. (2009). Psychiatric diagnosis and antiretroviral adherence among adolescent Medicaid beneficiaries diagnosed with human immunodeficiency virus/acquired immunodeficiency syndrome. *The Journal of Nervous and Mental Disease*, 197(5), 354-361.

Walther, D. J., Peter, J. U., Bashammakh, S., Hörtnagl, H., Voits, M., Fink, H., & Bader, M. (2003). Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science*, 299(5603), 76-76.

Wang, C., & Meier, U. T. (2004). Architecture and assembly of mammalian H/ACA small nucleolar and telomerase ribonucleoproteins. *The EMBO Journal*, 23(8), 1857-1867.

Wang, W., Wang, L., Xu, H., Cao, C., Liu, P., Luo, S., ... & Zhang, X. (2019). Characteristics of pro-and anti-inflammatory cytokines alteration in PTSD patients exposed to a deadly earthquake. *Journal of Affective Disorders*, 248, 52-58.

Wang, Y., Karstoft, K. I., Nievergelt, C. M., Maihofer, A. X., Stein, M. B., Ursano, R. J., ... & Werge, T. (2019). Post-traumatic stress following military deployment: Genetic associations and cross-disorder genetic correlations. *Journal of Affective Disorders*, 252, 350-357.

Wang, Y., Sun, N., Liu, Z., Li, X., Yang, C., & Zhang, K. (2016). Psychosocial mechanisms of serotonin transporter's genetic polymorphism in susceptibility to major depressive disorder: Mediated by trait coping styles and interacted with life events. *American Journal of Translational Research*, 8(2), 1281.

Ward Jr, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American statistical association*, 58(301), 236-244.

Watson, J. D. (1972). Origin of concatemeric T7DNA. Nature New Biology, 239(94), 197-201.

Weissman, M. M., Wickramaratne, P., Merikangas, K. R., Leckman, J. F., Prusoff, B. A., Caruso, K. A., . . . Gammon, G. D. (1984). Onset of major depression in early adulthood: Increased familial loading and specificity. *Archives of General Psychiatry*, 41(12), 1136-1143.

Stellenbosch University https://scholar.sun.ac.za

Weitkamp, K., Daniels, J. K., Romer, G., & Wiegand-Grefe, S. (2013). Health-related quality of life of children and adolescents with mental disorders. *Health and quality of life outcomes*, 11(1), 129.

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., ... & Burstein, R. (2013). Global burden of disease attributable to mental and substance use disorders: Findings from the Global Burden of Disease Study 2010. *The Lancet*, 382(9904), 1575-1586.

Wickramaratne, P. J., & Weissman, M. M. (1998). Onset of psychopathology in offspring by developmental phase and parental depression. *Journal of the American Academy of Child & Adolescent Psychiatry*, 37(9), 933-942.

Wikgren, M., Maripuu, M., Karlsson, T., Nordfjäll, K., Bergdahl, J., Hultdin, J., ... & Norrback, K. F. (2012). Short telomeres in depression and the general population are associated with a hypocortisolemic state. *Biological Psychiatry*, 71(4), 294-300.

Willis, A. M., & Brock, M. S. (2019). Adverse childhood experiences and serotonin transporters: A gene environmental study of the risk of PTSD in Soldiers (ACES). 59th Medical Wing Science and Technology JBSA United States.

Wojcicki, J. M., Shiboski, S., Heyman, M. B., Elwan, D., Lin, J., Blackburn, E., & Epel, E. (2016). Telomere length change plateaus at 4 years of age in Latino children: Associations with baseline length and maternal change. *Molecular Genetics and Genomics*, 291(3), 1379-1389.

Wolkowitz, O. M., Mellon, S. H., Epel, E. S., Lin, J., Dhabhar, F. S., Su, Y., ... & Compagnone, M. (2011a). Leukocyte telomere length in major depression: Correlations with chronicity, inflammation and oxidative stress-preliminary findings. *PloS One*, 6(3), e17837.

Wolkowitz, O. M., Mellon, S. H., Epel, E. S., Lin, J., Reus, V. I., Rosser, R., ... & Blackburn, E. H. (2012). Resting leukocyte telomerase activity is elevated in major depression and predicts treatment response. *Molecular Psychiatry*, 17(2), 164.

Wolkowitz, O. M., Reus, V. I., & Mellon, S. H. (2011b). Of sound mind and body: Depression, disease, and accelerated aging. *Dialogues in Clinical Neuroscience*, 13(1), 25.

Wong, J. Y., De Vivo, I., Lin, X., Fang, S. C., & Christiani, D. C. (2014). The relationship between inflammatory biomarkers and telomere length in an occupational prospective cohort study. *PloS One*, 9(1), e87348.

Stellenbosch University https://scholar.sun.ac.za

Woodward, L. J., & Fergusson, D. M. (2001). Life course outcomes of young people with anxiety disorders in adolescence. *Journal of the American Academy of Child & Adolescent Psychiatry*, 40(9), 1086-1093.

Woollett, N., Cluver, L., Bandeira, M., & Brahmbhatt, H. (2017). Identifying risks for mental health problems in HIV positive adolescents accessing HIV treatment in Johannesburg. *Journal of Child & Adolescent Mental Health*, 29(1), 11-26.

World Health Organization (2017). Depression and other common mental disorders: Global health estimates. No. WHO/MSD/MER/2017.2. World Health Organization.

Wray, N. R., Ripke, S., Mattheisen, M., Trzaskowski, M., Byrne, E. M., Abdellaoui, A., ... & Bacanu, S. A. (2018). Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature Genetics*, 50(5), 668.

Xavier, G., Spindola, L. M., Ota, V. K., Carvalho, C. M., Maurya, P. K., Tempaku, P. F., ... & Miguel, E. C. (2018). Effect of male-specific childhood trauma on telomere length. *Journal of Psychiatric Research*, 107, 104-109.

Xiao, Y., Liu, D., Liu, K., Wu, C., Zhang, H., Niu, Y., & Jiang, X. (2019). Association of DRD2, 5-HTTLPR, and 5-HTTVNTR gene polymorphisms with posttraumatic stress disorder in Tibetan adolescents: A case–control study. *Biological Research for Nursing*, 21(3), 286-295.

Xie, P., Kranzler, H. R., Poling, J., Stein, M. B., Anton, R. F., Brady, K., ... & Gelernter, J. (2009). Interactive effect of stressful life events and the serotonin transporter 5-HTTLPR genotype on posttraumatic stress disorder diagnosis in 2 independent populations. *Archives of General Psychiatry*, 66(11), 1201-1209.

Yu, R., & Woo, J. (2016). Exploring the link between depression and accelerated cellular aging: Telomeres hold the key. *Research and Reports in Biochemistry*, 6, 1-12.

Zalsman, G., Huang, Y. Y., Oquendo, M. A., Burke, A. K., Hu, X. Z., Brent, D. A., ... & Mann, J. J. (2006). Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *American Journal of Psychiatry*, 163(9), 1588-1593.

Stellenbosch University https://scholar.sun.ac.za

Zanet, D. L., Thorne, A., Singer, J., Maan, E. J., Sattha, B., Le Campion, A., ... & Côté, H. C. (2014). Association between short leukocyte telomere length and HIV infection in a cohort study: No evidence of a relationship with antiretroviral therapy. *Clinical Infectious Diseases*, 58(9), 1322-1332.

Zaubler, T. S., & Katon, W. (1996). Panic disorder and medical comorbidity: A review of the medical and psychiatric literature. *Bulletin of the Menninger Clinic*, 60(2).

Zhang, L., Hu, X. Z., Benedek, D. M., Fullerton, C. S., Forsten, R. D., Naifeh, J. A., ... & Le, T. (2014). The interaction between stressful life events and leukocyte telomere length is associated with PTSD. *Molecular Psychiatry*, 19(8), 855-856.

Zhang, X., Gainetdinov, R. R., Beaulieu, J. M., Sotnikova, T. D., Burch, L. H., Williams, R. B., ... & Caron, M. G. (2005). Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron*, 45(1), 11-16.

Zhao, J., Miao, K., Wang, H., Ding, H., & Wang, D. W. (2013). Association between telomere length and type 2 diabetes mellitus: A meta-analysis. *PloS One*, 8(11), e79993.

Zill, P., Baghai, T., Zwanzger, P., Schüle, C., Eser, D., Rupprecht, R., . . . Ackenheil, M. (2004). SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. *Molecular Psychiatry*, 9(11), 1030.

Zlotorynski, E. (2019). Telomere crisis activates autophagic death. *Nature Reviews Molecular Cell Biology*, 20(3), 133.

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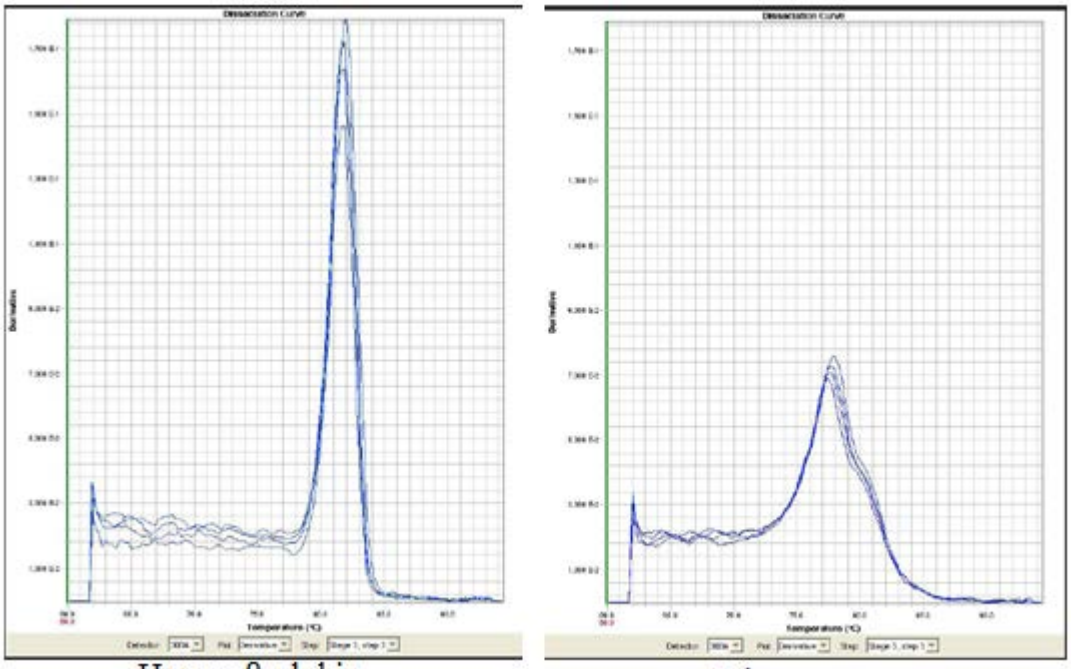
ADDENDUM A



Human β-globin Telomere

Dissociation curves for the optimized telomere and human β-globin assays

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ADDENDUM B

frontiers in Genetics

ORIGINAL RESEARCH
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Internalizing Mental Disorders and Accelerated Cellular Aging Among Perinatally HIV-Infected Youth in Uganda

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Introduction: Internalizing mental disorders (IMDs) in HIV+ children and adolescents are associated with impaired quality of life and non-adherence to anti-retroviral treatment. Telomere length is a biomarker of cellular aging, and shorter telomere length has been associated with IMDs. However, the nature of this association has yet to be elucidated.

Objective: We determined the longitudinal association between IMDs and relative telomere length (rTL) and the influence of chronic stress among Ugandan perinatally HIV-infected youth (PHIY).

Methods: IMDs (depressive disorders, anxiety disorders, and post-traumatic stress disorder) and IMDs were assessed using the locally adapted Child and Adolescent Symptom Inventory-5. In 368 PHIY with any IMD and 368 age- and sex-matched PHIY controls without any psychiatric disorder, rTL was assessed using quantitative polymerase chain reaction. Hierarchical cluster analysis was used to generate the three chronic stress classes (mild, moderate, and severe). *t*-tests were used to assess the difference between baseline and 12 month rTL and the mean difference in rTL between cases and controls both at baseline and at 12 months. Linear regression analysis was used to model the effects of chronic stress on the association between IMDs and rTL, controlling for age and sex.

Results: We observed longer rTL among cases of IMDs compared with controls ($p < 0.001$). We also observed a statistically significant reduction in rTL between baseline and 12 months in the combined sample of cases and controls ($p < 0.001$). The same statistical difference was observed when cases and controls were individually analyzed ($p < 0.001$). We found no significant difference in rTL between cases and controls at 12 months ($p = 0.117$). We found no significant influence

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of chronic stress on the association between IMDs and rTL at both baseline and 12 months.

Conclusion: rTL is longer among cases of IMDs compared with age- and sex-matched controls. We observed a significant attrition in rTL over 12 months, which seems to be driven by the presence of any IMDs. There is a need for future longitudinal and experimental studies to understand the mechanisms driving our findings.

Keywords: internalizing mental disorders, relative telomere length, HIV+, perinatally HIV-infected youth, Uganda

BACKGROUND

Human immunodeficiency virus/acquired immunodeficiency disease syndrome (HIV/AIDS) is a significant global health burden, with approximately 36.9 million people infected globally (UNAIDS, 2018). Both eastern and southern Africa remain the most affected regions, accounting for 45% of the world's HIV infections (UNAIDS, 2018). Of the over 2 million HIV-positive (HIV+) children globally, 90% reside in sub-Saharan Africa (UNAIDS, 2010). In Uganda, the country with the fifth-highest HIV prevalence in the region, an HIV prevalence of 0.5% has been reported among children aged 0–14, which corresponds to approximately 95,000 children living with HIV in the country (UPHIA, 2016–2017). The introduction of antiretroviral therapy (ART) has led to improved survival of HIV-infected youth (7–17 years); however, the mental health of these youth has received less attention (Mupambireyi et al., 2014). Perinatally HIV-infected youth (PHIY) are faced with a burden of psychiatric morbidity (Kamau et al., 2012), in addition to delayed motor and cognitive development (Le Doaré et al., 2012; Van Rie et al., 2007). Studies undertaken in both the developed (Europe and the United States) and developing world (sub-Saharan Africa) have documented depression rates of between 12.7% and 40% (Musisi and Kinyanda, 2009; Gadow et al., 2012; Kamau et al., 2012; Mellins et al., 2012; Nachman et al., 2012; Lwidiko et al., 2018; Kim et al., 2014) among PHIY. For anxiety disorders, rates of 9% to 32.2% have been reported among PHIY (Kamau et al., 2012; Mellins et al., 2012; Nachman et al., 2012; Kinyanda et al., 2019).

IMDs are associated with psychological distress (Musisi and Kinyanda, 2009), impaired quality of life, and non-adherence to ART (Walkup et al., 2009; Malee et al., 2011). In addition, patients with IMDs have higher mortality rates than have the general population (Cuijpers and Smit, 2002; Colton and Manderscheid, 2006; Ahmadi et al., 2011; Druss et al., 2011).

IMDs are characterized by quiet, internal distress (Tandon et al., 2011), in contrast to externalizing disorders, where overtly socially negative or disruptive behavior is displayed (Tandon et al., 2011). IMDs with high levels of negative affectivity include depressive disorders (e.g., dysthymic disorder), anxiety disorders (e.g., generalized anxiety disorder and social anxiety disorder), and obsessive-compulsive disorder (Regier et al., 2013; Turygin et al., 2013). Despite intensive research, the diagnosis of IMDs is still largely based on clinical symptoms, with an absence of biological markers to facilitate diagnosis. This is largely because the pathophysiological mechanisms underlying IMDs, such as depression and anxiety, are still largely unknown. Several studies have investigated the association between telomere length (TL) and IMDs, and shorter TL has been reported in adults with depression (Simon et al., 2006; Verhoeven et al., 2014; Cai et al., 2015) and anxiety disorders (Kananen et al., 2010; Verhoeven et al., 2015).

Telomeres are protein-bound deoxyribonucleic acid (DNA) repeat structures at the ends of chromosomes (Lindqvist et al., 2015), and are important in preventing chromosomes from fusing together during mitosis, thus preventing loss of genetic data (Allsopp et al., 1992; Blackburn et al., 2006). They also regulate cellular replicative capacity (Allsopp et al., 1992; Blackburn et al., 2006). During somatic cell replication, telomeres progressively shorten due to the inability of DNA polymerase enzyme to fully replicate the 3′ end of the DNA strand (Allsopp et al., 1992; Blackburn et al., 2006), a process termed as the "end replication problem" (Watson, 1972). This results in a gradual decline in telomere length (TL) over time. Once a critically short TL is reached, the cell is triggered to enter replicative senescence and subsequently cell death (Allsopp et al., 1992; Blackburn et al., 2006). TL provides a metric of cellular age and accounts for roughly 15% of the variance of age (Epel and Prather, 2018). TL has been reported to shorten in a predictable way with chronological age by roughly 20–40 base pairs per year (Cesare and Reddel, 2010). TL is partially genetically determined, with heritability estimates ranging from 36% to 84% (Aviv, 2012) and is highly variable between individuals (Vasa-Nicotera et al., 2005; Njajou et al., 2007). The current study assessed TL as relative TL (rTL), with rTL being proportional to an individual's TL (Cawthon, 2009).

Abbreviations: µL, microliter; ART, anti-retroviral therapy; CASI-5, Child and Adolescent Symptom Inventory—edition 5; CD4, cluster of differentiation 4; CIs, confidence intervals; Ct, threshold cycle; DNA, deoxyribonucleic acid; DSM-5, *Diagnostic Statistical Manual for Mental Disorders*—edition 5; HBG, human β-globin gene; HCA, hierarchical cluster analysis; HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency disease syndrome; HIV+, HIV positive; IMD, internalizing mental disorder; JCRC, Joint Clinical Research Centre; LTL, leucocyte telomere length; MRC/DfID, Medical Research Council/Department for International Development; ng, nanogram; PTSD, post-traumatic stress disorder; qPCR, quantitative polymerase chain reaction; rTL, relative telomere length; s, second; scg, single copy gene; TASO, The AIDS Support Organization; TERC, telomerase RNA complex; TERT, telomerase reverse transcriptase; TL, telomere length; UNAIDS, The Joint United Nations Programme on HIV and AIDS; UVRI, Uganda Virus Research institute.

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Several studies in youth have reported associations between adversity and telomere shortening (Shalev et al., 2013; Theall et al., 2013; Drury et al., 2014; Mitchell et al., 2014). Adversity experienced in youth ranges from exposure to traumatic stressors, such as sexual and physical abuse, to social adversities that relate to family structure, parental mental distress, and socio-economic status (SES). Causal associations between stressful life events and early adversities, such as childhood sexual abuse and major depression, are well documented (Kendler et al., 1999; Fergusson and Mullen, 1999; Kendler et al., 2000), with evidence suggesting molecular signatures of stress overlap with major depression (Cai et al., 2015) Biological processes, such as inflammation and oxidative stress, which have been observed in several psychiatric disorders are also associated with telomere shortening (Wolkowitz et al., 2011a; Wolkowitz et al., 2011b), suggesting that telomere shortening may be related to certain psychiatric endophenotypes.

Depression has been considered a syndrome of accelerated aging (Heuser, 2002). The first study to examine leucocyte TL (LTL) in a group of subjects with either major depression or bipolar disorder and aged-matched controls found shorter LTL among cases compared with healthy controls (Simon et al., 2006). A large longitudinal clinical cohort study found shorter LTL among groups who were currently depressed or had remitted depression compared with healthy controls (Verhoeven et al., 2014). However, there was no statistically significant difference in LTL between the currently depressed and remitted depression groups, suggesting that depression may leave an "indelible marker" on LTL. However, in the currently depressed group, a dose–response relationship was observed, with LTL inversely associated with both severity and duration of depression. This dose–response relationship was further supported by a longitudinal study by Shalev et al. (2014), where persistence of IMDs from 11 to 38 years predicted reduced LTL at 38 years of age in a dose-dependent manner among male participants. It is, however, not possible to rule out that LTL was already reduced at the first episode of depression, indicating that shorter LTL could be a risk factor for depression. Indeed, Gotlib et al. (2015) described shorter LTL as a risk marker for depression, where shorter LTL was observed among girls (aged 10–14 years) at increased risk for depression. High risk for depression was assessed as having a mother with a history of recurrent episodes of depression, while low risk was assessed as having a mother with no current or past Axis I disorder during a girl's lifetime. However, results across studies have been inconsistent. While several other studies have reported shorter LTL among currently depressed individuals compared with controls (Lung et al., 2007; Hoen et al., 2011; Wikgren et al., 2012; Garcia-Rizo et al., 2013), some studies have failed to find an association (Wolkowitz et al., 2011a; Teyssier et al., 2012; Needham et al., 2015; Schaakxs et al., 2015).

Accelerated aging has also been described in anxiety disorders. Using the same study population as described in Verhoeven et al. (2014), the authors reported shorter LTL among subjects with a diagnosis of current anxiety disorder than among controls (Verhoeven et al., 2015). There was, however, no statistically significant difference in LTL between the remitted anxiety disorder group and controls, suggesting that LTL shortening in anxiety disorders may be more reversible than that associated with depression. Needham et al. (2015) reported an association between shorter LTL and a diagnosis of generalized anxiety disorder and panic disorder among women. Kananen et al. (2010) reported shorter LTL among older anxiety disorder subjects (48–87 years of age) compared with controls, and a study by Okereke et al. (2012) reported a dose–response relationship where severe phobia was associated with shorter LTL.

PTSD has also been considered in the context of accelerated aging (Moreno-Villanueva et al., 2013; Miller and Sadeh, 2014). Shorter LTL has been implicated in PTSD, though the effects were primarily explained by early life stress (O'Donovan et al., 2011). Shorter LTL was reported among combat-deployed soldiers with PTSD, compared with those without PTSD (Zhang et al., 2014). There is a need to understand whether telomere shortening is a direct effect of PTSD, whether the development of PTSD and shortening of telomeres are simultaneous effects of increased stress reactivity (Zhang et al., 2014), or whether telomere shortening is a risk factor for PTSD (Malan et al., 2011).

HIV infection has also been found to be associated with shortened telomeres (Oeseburg et al., 2010; Auld et al., 2016). HIV/AIDS may be viewed as a chronic psychological stressor due to the illness and stigma that are associated with the disease (Varni et al., 2012). Since TL has been found to be a marker for chronic stress (Needham et al., 2015), shorter telomeres are expected in HIV/AIDS subjects as compared with the disease-free population.

We hypothesized that in PHIY in Uganda, attrition in rTL over a 12-month period would be greater in cases of IMDs compared with age- and sex-matched controls without any psychiatric disorder. We further hypothesized that cases would have shorter rTL than controls. We thus aimed to determine the longitudinal association between IMDs and rTL and the influence of chronic stress in this relationship.

METHODS

Study Design

This case–control study was nested within a Medical Research Council/Department for International Development (MRC/DfID)-funded project that investigated mental health among children and adolescents living with HIV/AIDS in Kampala and Masaka districts of Uganda (CHAKA study), which enrolled 1,339 Ugandan PHIY (7–17 years) of black African ancestry (Kinyanda et al., 2019). All participants with any of the IMDs (368 cases) and an equal number of age- and sex-matched controls were selected from CHAKA (N = 736) and included in the present study. Both the baseline and 12-month archived blood sample for each of the included participants was retrieved from which genomic DNA was extracted.

Study Population

Study participants were recruited from two HIV clinics in urban Kampala [Joint Clinical Research Centre (JCRC) and Nsambya Home Care] and three HIV clinics in rural Masaka [The AIDS Support Organization (TASO), Kitovu Mobile Clinic, and Uganda Cares]. All study participants were on ART.

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Procedures

Consenting PHIY, as well as their caregivers, were interviewed using a structured questionnaire. The questionnaire included, among others, socio-demographic characteristics (sex, study site, age, caregiver level of education, and SES), and modules on depression, post-traumatic stress disorder, and anxiety modules from the DSM-5 referenced Children and Adolescent Symptom Inventory-5 (CASI-5) (Gadow, 2013). The CASI-5 was locally adapted for use in Uganda (Mpango et al., 2017). Trained psychiatric nurses and psychiatric clinical officers administered the CASI-5 at two time points (baseline and 12 months). The CASI-5 lists the symptoms of a wide range of psychiatric disorders including major depressive disorder, generalized anxiety disorder, PTSD, and attention-deficit/hyperactivity disorder, among others. Individual CASI-5 items are rated on a 4-point frequency of occurrence scale ranging from never (0) to very often (3). There are several CASI-5 scoring algorithms; however, in the present study we used symptom count cutoff scores that reflect the prerequisite number of symptoms for a clinical diagnosis. At each study visit, 4 ml of blood was withdrawn from each study participant through venipuncture into an EDTA vacutainer and was stored at −80 °C pending DNA extraction.

Inclusion and Exclusion Criteria

Inclusion criteria: i) HIV-infected outpatients, registered with any of the HIV clinics at any of the study sites; ii) aged between 7 and 17 years at the time of enrolment; iii) conversant in English or Luganda, the language into which the research assessment tools were translated; and iv) able to provide written informed consent(caregivers)/assent(adolescents). Cases were subjects who had any depressive disorder [depression or dysthymia (persistent depressive disorder)] or anxiety disorder. Controls were age-and sex-matched without any psychiatric disorder. Persistent IMDs were baseline cases that remained cases at 12 months, while remitted ones were baseline cases that lost disease status at 12 months

Exclusion criteria: i) Seriously ill including being unable to understand study procedures and ii) any other psychiatric disorder other than the ones listed above.

Ethical Considerations

Both CHAKA and the present study were conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The CHAKA study obtained ethical and scientific clearance from the Uganda Virus Research Institute (UVRI) Science and Ethical Committee (#GC/127/15/06/459) and the Uganda National Council of Science and Technology (#HS 1601). The present study obtained approval from the Higher Degrees Research & Ethics Committee, School of Biomedical Sciences, College of Health Sciences, Makerere University (#SBS 421) and the Health Research Ethics Committee of Stellenbosch University (#S17/09/179). Caregiver provided informed consent for their children/adolescents to participate in the study and for a blood specimen to be drawn for genetics analyses. Adolescents provided further assent to participate in the study. Study participants who were diagnosed with significant psychiatric problems were referred to mental health units at Entebbe and Masaka government hospitals.

Measure of Chronic Stress

Chronic stress was measured as social disadvantage and variables that were considered to confer social disadvantage were used to construct a composite index for chronic stress. A composite index of chronic stress was constructed from data collected on the following variables: orphanhood (double orphanhood carried a higher chronic stress score vs. single or not orphaned); food availability (not enough food carried a higher chronic stress score vs. enough food); study site (urban carried a higher chronic stress score than rural); and caregiver level of education (no formal education carried a higher chronic stress scores than primary and primary a higher stress score than secondary, etc.). Hierarchical cluster analysis (HCA) was used to generate the different cutoff points for each chronic stress class.

Chronic Stress Classes

The chronic stress index ranged from 0 to 3.75, with a normal distribution. A total of three chronic stress classes were generated during HCA, i.e., mild, moderate, and severe. The mild class had a chronic stress score of 0 to 1.375, the moderate class had a score of greater than 1.375 to 2.375, and the severe class had a score of greater than 2.375.

Analysis of Relative Telomere Length

DNA was extracted from blood collected from each participant, using the QiAmp Mini DNA Extraction Kit (Qiagen GmbH, Germany). Extracted DNA was quantified by 260/280 and 260/230 ultraviolet spectrophotometry on the NanoDrop 1000 spectrophotometer V3.7 (Thermo Fisher Scientific, Wilmington, MA). The DNA was subsequently diluted to 5 ng/μl and amplified using the KAPA SYBR FAST qPCR Master Mix (Merck, Darmstadt, Germany) per Cawthon et al. (2002), with slight modifications. Primers specific for telomeric repeats (T) (Cawthon, 2002) and a stably expressed single copy reference gene (S), the human β-globin (HBG1, 5′-GCTTCTGACACAACTGTGTTCACTAGC-3′; and HBG2, 5′-CACCAACTTCATCCACGTTCACC-3′), were used to amplify telomeric repeats and human β-globin, respectively. For the telomere assay, each reaction included 5 μl of KAPA SYBR FAST qPCR Master Mix (Merck, Darmstadt, Germany); 1.35 and 4.50 μM of forward and reverse primers, respectively; 5 ng of genomic DNA; and water in a 10-μl reaction volume. The human β-globin assay was identical to the telomere assay except that 2.0 μM of each of the forward and reverse primers were used. The reactions for the telomeric repeats and the human β-globin gene were amplified on the same 384-well plates. Each participant's DNA sample was amplified in triplicate. If the threshold cycle (Ct) values of the triplicates of particular samples differed by more than 0.5, those samples were excluded. From the triplicate Ct values, the means were calculated for each sample and used in subsequent calculations. Amplification was performed on the

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ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA) using the following thermal cycling profile: 95° C for 3 min, followed by 40 cycles of 95° C for 3 s and 60° C for 30 s, and a dissociation stage of 95° C for 15 s, 64° C for 15 s, and 95° C for 15 s. A calibrator sample was prepared by pooling equal amounts of DNA from each participant for the construction of a standard curve. The calibrator DNA sample was serially diluted 1.68-fold per dilution, to produce a nine-point standard curve, with DNA amounts ranging from 50 to 0.79 ng/μl. After amplification of the serial dilutions, a linear plot of the Ct versus the log value of the input amount of DNA (standard curve) was constructed using ABI's SDS v.2.3 software. The efficiency of a reaction was also determined from the standard curve of that reaction. Threshold and baseline values were used as determined by the SDS v.2.3 software. All Ct values were corrected for the PCR efficiency, and interplate calibrations were performed using GenEx software (http://www.gene-quantification.de/datan.html).

A validated qPCR method (Cawthon, 2002) was used to determine relative TLs (rTLs) in all samples. First, the mean telomere repeat copy number (tel, T) was normalized to a reference gene (single copy gene) (scg, S) copy number to control for differences in DNA quantity. The T/S ratio is proportional to the average TL. Thereafter, the factor by which the T/S ratio differs between the experimental sample and the calibrator sample is determined to provide an indication of relative average TL:

$$T/S = 2^{-\Delta Ct}$$

$$\text{Relative average TL(tel)} = 2^{-\Delta\Delta Ct}$$

$$\text{where } \Delta Ct = Ct(tel) - Ct(scg).$$

A T/S > 1 indicates that the average rTL in the sample is greater than that of the reference sample, and a T/S < 1 indicates that the average rTL in the experimental sample is less than that in the reference sample.

Power for the Study

We calculated the *post hoc* power for our study based on results from a study by Epel et al. (2004). We used the formula of sample size and power for difference in means in case–control studies. We worked on the assumption that cases (individuals with IMDs) would have higher levels of stress than controls (individuals without IMDs). Epel et al. (2004) found a 15% reduction in mean rTL among cases compared with controls. Given a 1:1 ratio of cases to controls and using a 5% level of significance, with 368 cases and controls, our study was well powered (power greater than 80%) to detect any reduction above 4.75% in mean rTL between cases and controls. For instance, a reduction of 5% in mean rTL between cases and controls provided a power of 83.8%.

Statistical Methods

Statistical analyses were conducted using Stata 15 (StataCorp, TX, USA).

Socio-demographic characteristics were described between cases and controls. Chi-square tests were used to assess the association between the socio-demographic characteristics and IMDs at baseline (cases vs. controls). SES was generated from a scale of nine household items (car, motorcycle, refrigerator, electricity, bicycle, radio, telephone, cupboard, and flask). Each item was weighted in the respective order, a car carrying a maximum weight of 9 and a flask a minimum weight of 1. A total score of items was generated, whose median cutoff of 13 was used to classify low and high SES. A score less than 13 was classified as low SES, while that greater than 13 was classified as high SES. Our study group (Kinyanda et al., 2011) has previously used household items as a measure of SES in rural settings of Uganda. A *t*-test was used to compare CD4 counts between cases and controls to account for any disparity in HIV disease progression.

Outliers were revealed by box and whisker plots and were all removed from the rTL data. The skewed rTL data became normally distributed after removal of outliers.

The distributions of rTL at baseline and at 12 months and the change in rTL were determined using a standardized normal probability plot (P-P plot) (See **Supplementary Materials**). The difference in rTL distribution at baseline and at 12 months was assessed using *t*-tests. The mean difference in rTL between cases and controls was also assessed using *t*-tests. One-way analysis of variance was used to assess whether there were any statistically significant differences between change in rTL and each of the variables of age, sex, study site, caregiver education level, and child education level.

Linear regression was used to i) assess the relationship between rTL and chronic stress, adjusting for sex and age; ii) model the effect of chronic stress on the association between IMDs and rTL, by comparing models without chronic stress to models with chronic stress; and iii) model the effect of age on the relationship between IMDs and rTL. There were missing data for rTL values at baseline or 12 months or both. For all analyses that needed computation of confidence intervals, we computed 95% confidence intervals; statistical significance was set at a *p*-value less or equal to 0.05, while a *p*-value greater than 0.05 but less than 0.07 was considered a trend towards marginal significance.

RESULTS

Socio-demographic factors were evenly distributed between cases and controls as shown in **Table 1**.

Tests of association between different socio-demographic variables and rTL were run to determine potential confounders (**Table 2**). None of the socio-demographic variables were associated with rTL. Study site, age, and SES were significantly associated with chronic stress ($p < 0.001$, $p = 0.040$, and $p = 0.015$, respectively).

Difference in rTL Between Cases and Controls

rTL was normally distributed at both baseline and 12 months. Mean rTL (95%CI) of the combined sample of cases and controls was 1.148 (1.119–1.176) at baseline and 0.905 (0.879–0.931) at 12 months. For cases, mean rTL (95%CI) was 1.198 (1.157–1.239) at baseline and 0.925 (0.886–0.965) at 12 months; while for

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TABLE 1 | Distribution of socio-demographic factors between cases and controls.

Variable (*n*)	Case *n* (%)	Control *n* (%)	95% confidence level
Sex			$p = 0.111$
Male (342)	160 (46.8)	182 (53.2)	
Female (393)	207 (52.7)	186 (52.7)	
Study site			$p = 0.941$
Urban (415)	208 (50.1)	207 (49.9)	
Rural (321)	160 (49.8)	161 (50.2)	
Age			$p = 0.374$
7–11 years (389)	202 (51.9)	187 (48.1)	
12–17 years (307)	149 (48.5)	158 (51.5)	
Education level (caregiver)			$p = 0.371$
No formal education (13)	9 (69.2)	4 (30.8)	
Primary (648)	323 (49.9)	325 (50.1)	
Secondary (35)	35/72 (48.6)	37/72 (51.4)	
SES			$p = 0.459$
Low (332)	171/332 (51.5)	161/332 (48.5)	
High (404)	197/404 (48.8)	207/404 (51.2)	
Mean CD4 count at baseline			
	947.04	944.02	$p = 0.939$

CD4, cluster of differentiation 4; primary, 0–7 years of formal education; secondary, 8–14 years of formal education; low SES, 0–13; high SES, > 13. All numbers that do not add up were due to missing data.

TABLE 2 | *p*-values for tests of association between socio-demographic variables and rTL change and chronic stress.

Variable	*p*-value (rTL change)	*p*-value (chronic stress)
Sex	0.298	0.122
Study site	0.235	<0.001
Age	0.553	0.040
Caregiver level of education	0.912	0.587
SES	0.897	0.015
Child level of education	0.611	0.364

SES, socioeconomic status.

controls, mean rTL (95%CI) was 1.097 (1.057–1.137) at baseline and 0.884 (0.851–0.917) at 12 months.

At baseline, we found a statistically significant difference in rTL between cases and controls ($p < 0.001$). However, contrary to what we expected, rTL was longer in cases compared with controls. There was, however, no statistical difference in rTL between cases and controls at 12 months ($p = 0.117$). In addition, the change between baseline and 12-month rTL (rTL change) did not differ statistically between cases and controls ($p = 0.608$) (**Table 3**).

Differences Between Baseline and 12-month rTL

In the combined analysis of baseline cases and controls there was significant attrition in rTL between baseline and 12 months ($p < 0.001$). This attrition did not differ by internalizing mental disorder (IMD) status ($p = 0.608$). A further stratified analysis of cases only and controls only yielded similar *p*-values of <0.001 (**Table 4**).

Association Between Chronic Stress and rTL

We observed a trend towards statistical significance between chronic stress and baseline rTL ($p = 0.067$). Severe stress was significantly associated with longer rTL ($p = 0.028$) (**Table 5**). However, chronic stress was not significantly associated with either 12-month rTL or a change in rTL ($p = 0.147$ and $p = 0.455$, respectively) (**Table 5**).

Association Between Chronic Stress and IMDs

We found a trend toward statistical significance between chronic stress and IMDs (**Table 6**).

The rTL and IMDs After 12 Months

We found no significant difference in baseline rTL between cases of IMDs that persisted compared to those that remitted after 12 months ($p = 0.235$). We also found no statistically significant association between 12-month rTL and 12-month IMD status ($p = 0.090$), as well as no association between disease severity and rTL at baseline ($p = 0.238$) and 12 months ($p = 0.264$).

Effect of Chronic Stress on the Association Between IMDs and rTL

We found no significant influence of chronic stress on the association between IMDs and rTL both at baseline and at 12 months (**Table 7**).

TABLE 3 | Difference in rTL between cases and controls.

Time point	Group	Obs	Mean rTL	Std. Dev	95%CI	*p*-value
Baseline	Cases	307	1.198	0.364	1.157–1.239	
	Controls	306	1.097	0.354	1.057–1.137	<0.001
12 months	Cases	278	0.925	0.336	0.886–0.965	
	Controls	274	0.884	0.275	0.851– 0.917	0.117
	Cases	231	–0.256	0.473	0.194–0.317	
rTL change	Controls	234	–0.234	0.416	0.181–0.288	0.608

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TABLE 4 | Differences between baseline and 12 months rTL for baseline cases and controls.

Group	Time point	Obs	Mean TL	Std. Dev	95% conf. interval	*p*-value
Total sample	Baseline	465	1.148	0.366	1.115–1.182	
	12 months	465	0.903	0.314	0.875–0.932	<0.001
Cases	Baseline	231	1.190	0.371	1.142–1.238	
	12 months	231	0.935	0.348	0.889–0.980	<0.001
Controls	Baseline	234	1.107	0.356	1.061–1.153	
	12 months	234	0.873	0.274	0.837–0.910	<0.001

TABLE 5 | Assessing association between chronic stress and rTL, adjusted for age and sex.

Time point	Chronic stress class	Coefficient	*p* > |*t*|	95% conf. interval	*p*-value
	Mild	Reference			
Baseline	Moderate	0.013	0.717	–0.055 to 0.080	0.067
	Severe	0.091	0.028	0.010 to 0.173	
	Mild	Reference			
12 month	Moderate	–0.031	0.288	–0.089 to 0.026	0.147
	Severe	0.034	0.334	–0.035 to 0.104	
	Mild	Reference			
rTL change	Moderate	0.058	0.214	–0.034 to 0.149	0.451
	Severe	0.037	0.507	–0.073 to 0.147	

Reference, reference chronic stress class during regression analysis.

TABLE 6 | Association between chronic stress and internalizing mental disorders.

Chronic stress class	Cases (*n*)	Controls (*n*)	Total	*p*-value
Mild	120	144	264	0.065
Moderate	178	147	325	
Severe	70	77	147	
Total	368	368	736	

Effect of Age on the Relationship Between IMDs and rTL

On stratifying our analyses for age [children (7–11 years) and adolescents (12–17 years)], we observed no statistically significant differences by age group for IMDs and rTL compared with those that were observed with both age categories combined (**Table 8**).

DISCUSSION

In this study, we investigated the association between chronic stress and rTL among PHIY cases with IMDs and age- and sex-matched controls in Uganda. To our knowledge, this is the first

TABLE 7 | Effect of chronic stress on the association between IMDs and rTL.

Baseline	Disease status	Coefficient	*p* > |*t*|	95% conf. interval	*p*-value	*R*-squared
Without CS	Controls	Reference			<0.001	
	Cases	0.101	0.001	0.044 to 0.15834		0.020
With CS	Controls	Reference				
	Cases	0.101	0.001	0.044 to 0.15796		
	Mild	Reference				
	Moderate	0.017	0.612	–0.048 to 0.081	<0.001	0.028
	Severe	0.088	0.028	0.010 to 0.167		
12 months	**Disease status**	**Coefficient**	***p*** **> |*t*|**	**95% conf. interval**	***p*-value**	***R*-squared**
Without CS	Controls	Reference				
	Cases	0.041	0.117	–0.010 to 0.092	0.117	0.005
With CS	Controls	Reference				
	Cases	0.040	0.128	0.012 to 0.091		
	Mild	Reference				
	Moderate	0.030	0.322	–0.087 to 0.029		
	Severe	0.035	0.316	0.034 to 0.105	0.128	0.011

CS, chronic stress; reference, reference disease status/chronic stress class during regression analysis.

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TABLE 8 | Effect of age on the association between IMDs and rTL.

Baseline					
Category	**IMDs status**	**Coefficient**	***p*** **> |t|**	**95% conf. interval**	***p*-value**
Total sample	Controls	Reference			
	Cases	0.101	0.001	0.044 to 0.158	<0.001
Children	Controls	Reference			
	Cases	0.094	0.025	0.012 to 0.175	0.025
Adolescents	Controls	Reference			
	Cases	0.110	0.007	0.031 to 0.190	0.007
12 months					
Category	**IMDs status**	**Coefficient**	***p*** **> |t|**	**95% conf. interval**	***p*-value**
Total sample w	Controls	Reference			
	Cases	0.041	0.117	−0.010 to 0.092	0.117
Children	Controls	Reference			
	Cases	0.048	0.181	−0.022 to 0.118	0.181
Adolescents	Controls	Reference			
	Cases	0.033	0.395	−0.043 to 0.109	0.395
rTL change					
Category	**IMDs status**	**Coefficient**	***p*** **> |t|**	**95% conf. interval**	***p*-value**
Total sample	Controls	Reference			
	Cases	0.021	0.608	−0.060 to 0.102	0.601
Children	Controls	Reference			
	Cases	0.017	0.768	−0.098 to 0.133	0.768
Adolescents	Controls	Reference			
	Cases	0.027	0.639	−0.086 to 0.140	0.639

Children, 7–11 years; adolescents, 12–17 years; reference, reference IMDs status during regression analysis.

sub-Saharan African study to investigate the association between chronic stress with rTL and IMDs among PHIY.

Several studies have determined the association between TL and different internalizing psychopathologies. Shorter TL have been reported among cases of depression compared with controls (Garcia-Rizo et al., 2013; Shalev et al., 2014; Verhoeven et al., 2014), while others have failed to find significant associations (Wolkowitz et al., 2011a; Teyssier et al., 2012; Simon et al., 2015). Shorter TL has also been implicated in both anxiety disorders (Kananen et al., 2010; Verhoeven et al., 2015) and PTSD (O'Donovan et al., 2011; Zhang et al., 2014) and has been reported to confer risk for PTSD (Malan et al., 2011). Due to these reported associations of shorter TL in the different internalizing psychopathologies, we hypothesized that rTL would be shorter among cases of IMDs than controls in our study participants. Contrary to our hypothesis, we observed longer rTL among cases of IMDs compared with their controls ($p < 0.001$). Longer rTL among IMDs could be due to elevated telomerase levels. TL is maintained by a telomerase enzyme component known as telomerase RNA component (TERC) and a reverse transcriptase enzyme known as the telomerase reverse transcriptase (TERT) (Wang and Meier, 2004; Blackburn et al., 2006). Wolkowitz et al. (2012) indeed reported elevated telomerase levels among people with depression than among healthy matched controls at baseline. After 8 weeks of treatment with selective serotonin re-uptake inhibitors, they found that telomerase levels became even more elevated as depression remitted. It has been speculated that elevated telomerase levels are a compensatory effort towards excessive loss of telomeres (Damjanovic et al., 2007; Lin et al., 2012).

We also observed a statistically significant reduction in rTL between baseline and 12 months in a combined sample of cases and controls ($p < 0.001$). A statistical difference was also observed when cases and controls were individually analyzed ($p < 0.001$). This difference was expected since TL generally decreases over the life span (Muezzinler et al., 2013). We found no significant difference in rTL between cases and controls at 12 months ($p = 0.117$). Since cases had significantly longer rTL than controls at baseline ($p < 0.001$), the lack of a significant difference at 12 months indicates greater rTL attrition among cases compared with controls. This is an interesting observation that points to the notion that IMDs are possibly driving accelerated cellular aging (rTL attrition). Indeed, telomere shortening has been reported to be strongly influenced by chronic stress exposure (Ridout et al., 2015), and suffering from a chronic disease, such as heart disease (Haycock et al., 2014) and diabetes (Zhao et al., 2013), has been conceptualized as a prolonged stress exposure that could explain their association with TL. IMDs have been reported as chronic stressors (McEwen, 2003) with chronic biological adaptations that result in long-term biological damage that could potentially explain rTL attrition due to IMDs. IMDs could also be leading to rTL attrition through inflammatory pathways. Depression has been reported to prime larger cytokine responses to stressors

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(Kiecolt-Glaser et al., 2015). Increased systemic inflammation has been associated with decreased TL among a prospective cohort of workers exposed to high level of fine particulate matter (Wong et al., 2014), while interventions that attenuate inflammatory processes in fear- and anxiety-based disorders have been thought to be effective in mitigating the symptoms of anxiety disorders (Michopoulos et al., 2017).

If IMDs were driving rTL attrition, we would expect significant reduction in rTL among cases with no corresponding significant reduction among controls. Intriguingly, we observed significant reduction in rTL in both groups ($p < 0.001$). This is possibly due to general longitudinal reduction in rTL. However, study subjects were only followed up for 12 months, and a longer follow-up period may be required to see a true difference in rTL attrition between cases and controls. It needs to be borne in mind that other factors may be responsible for either the overall greater reduction in rTL over 12 months or the greater rTL reduction among cases than controls. For example, participants were all on ART, with the type of ART regimen not accounted for in the analysis. Also, factors known to affect rTL, such as diet (Shiels et al., 2011) and frequency of physical exercise (Cherkas et al., 2008) were not accounted for. In addition, effects on rTL may have been determined even before birth from maternal stress, or through direct transmission of maternal rTL.

Although previous studies among children have found associations between TL and socio-demographic variables, such as caregiver level of education (Needham et al., 2012), parental SES (Needham et al., 2012; Mitchell et al., 2014), sex (Drury et al., 2014), and living environments (Theall et al., 2013), we found no association between any baseline socio-demographic variables and rTL change in the present study. This discrepancy could be due to cultural context, as previous studies were carried out in developed world settings that differ from the African low-income setting of this study. For example, stress due to orphanhood in the Ugandan context may be experienced differently, as there is a strong extended family system in Uganda where orphans tend to be taken care of by their uncles or aunts, unlike in the developed world where orphans are often institutionalized. The latter has been associated with shorter TL (Drury et al., 2012). More studies are needed to understand factors that affect TL in the sub-Saharan African context.

We found no association between rTL change and persistence or remission of IMDs. This further suggests that rTL does not drive IMDs, but rather IMDs may be driving accelerated cellular aging. Higher mortality rates have been reported among patients with IMDs compared with the general population, and the mortality is mainly due to the same age-related diseases as the general population, such as cancer, and heart, and cerebrovascular disease. For example, a study by Colton and Manderscheid (2006) reported that clients with a diagnosis of major mental illness died 1 to 10 years earlier than did clients with no major mental illness. Another study reported that persons with mental disorders died an average of 8.2 years younger than did the rest of the population and that presence of a mental illness was associated with a hazard ratio of 2 over a 17-year study period (Druss et al., 2011), supporting the mediating role of IMDs in accelerated cellular aging.

Psychological stress (both perceived stress and chronicity of stress) has been significantly associated with lower telomerase activity and shorter TL (Epel et al., 2004). We investigated the association between chronic stress and rTL in our sample. We observed a marginally significant association between chronic stress and rTL ($p = 0.067$). However, contrary to expectation, severe chronic stress was associated with longer rTL ($p = 0.028$) (**Table 5**). Longer rTL was also associated with IMD caseness. Thus, if increased stress (chronic) is an acquired vulnerability factor for IMDs, then it stands to reason that severe chronic stress would be associated with longer rTL, an association that we indeed observed. Further, since IMDs are associated with impaired quality of life and negative clinical and behavioral outcomes among PHIY and poor adherence to ART (Malee et al., 2011; Walkup et al., 2009), we expected significantly lower CD4 counts among cases than controls. However, we found no significant difference in mean CD4 count between cases and controls ($p = 0.939$). We did not investigate other virologic markers of HIV disease severity, such as viral load. However, all study participants were on ART, and thus no difference would be expected if adherence to treatment was similar between cases and controls.

We observed an association between chronic stress and study site and SES respectively. Living in urban areas and having a high SES were associated with more chronic stress than living in rural areas and having a low SES. The association of both urban location and high SES with increased chronic stress may be due to a correlation between the two variables, as participants in urban areas are often of higher SES as compared with their rural counterparts. The association of urban location with increased chronic stress could be due to ecological factors and pressures that are associated with urban life as compared with rural life. We also observed an association between age and chronic stress. Adolescents (12–17 years) were more stressed than children (7–11 years) and this could be due to the fact that adolescents were aware of their HIV status and the stress could be associated with the burden of being HIV+ and stigma among these study participants (Knizek et al., 2017).

Lastly, since severe chronic stress is associated with longer rTL, we expected severe chronic stress to lower the p-value of the regression for the association between IMDs abd rTL, an interaction we did not observe. We think that this could due to duration of chronic stress. Although the chronic stress variables used in the present study are known stressors in this population, the duration of the stressor was not assessed for.

LIMITATIONS AND RECOMMENDATIONS

We defined IMDs as having any depressive disorder or anxiety disorder or PTSD. The inclusion of PTSD is contentious as the disorder has recently been delineated from IMDs in the DSM-5 and may have skewed our findings. We recommend that future studies undertake a comparative analysis of the different disorders

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that make up the IMD spectrum to elucidate the independent contribution of each particular disorder.

We did not investigate factors that are known to affect rTL, such as frequency of physical activity, medication, diet, and presence of other comorbid diseases. Also, much as CD4 counts did not significantly differ between cases and controls, the ART regimen for each study participant was not accounted for in the analysis. Future studies should endeavor to consider these factors.

Both the duration and severity of IMDs have been shown to affect rTL. We did not assess the duration of IMDs. However, we think that this may not have greatly affected our findings because disease severity was not significantly associated with rTL in the present study. Future studies should, however, account for the duration of IMDs.

We suggest that the longer rTL observed among cases is due to elevated telomerase activity/levels. However, we did not investigate telomerase activity/levels between cases and controls. Future studies should investigate this possibility. Also, certain genes, such as the telomerase reverse transcriptase and telomerase RNA component, have been reported to influence TL biology. The role of polymorphisms in these genes influencing rTL needs to be investigated, and future studies should endeavor to address this.

Chronic stress was measured using a number of context-specific indicators because there is no locally adapted tool for assessing chronic stress in this setting. While this may be a limitation and may limit generalizability to other settings, the variables used to generate the chronic stress index are known stressors in this population. Validation of the chronic stress index tool will be required in future studies in Uganda.

CONCLUSIONS

RTL was longer in cases with IMDs compared with age- and sex-matched controls.

We observed significant attrition in rTL over 12 months. This rTL attrition seems to be driven by the presence of any IMDs, indicating that IMDs could be driving accelerated rTL attrition. Mechanisms that either directly influence rTL or alleviate the effects of IMDs on rTL attrition could explain our study findings, and longitudinal and experimental studies are needed to fully elucidate underlying mechanisms.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study obtained ethics approval from the Health Research Committee of Stellenbosch University (#S17/09/179) and the higher Degrees Research & Ethics Committee of the School of Biomedical Sciences, College of Health Sciences, Makerere University (#SBS 421). The parent study (CHAKA) obtained ethics approval from the Uganda Virus Research Institute's Science and Ethical Committee (#GC/127/15/06/459) and the Uganda National Council of Science and Technology (#HS 1601). All caregivers provided informed consent for their children/adolescents to participate in the study and for a blood specimen to be withdrawn from them (child/adolescent) for rTL and other genetics analyses. Adolescents further provided informed assent to participate in the study.

CONSENT FOR PUBLICATION

No details, images, or videos relating to any of the study participants are included in this manuscript.

ETHICS STATEMENT

The study obtained ethics approval from the Health Research Committee of Stellenbosch University (# S17/09/179) and the Higher Degrees Research & Ethics Committee, School of Biomedical Sciences, College of Health Sciences, Makerere University (# SBS 421). The parent study (CHAKA) obtained ethics approval from the Uganda Virus Research Institute (UVRI) Science and Ethical Committee (# GC/127/15/06/459) and the Uganda National Council of Science and Technology (# HS 1601). All study participants provided written informed consent/assent to participate in the study and for a blood specimen to be withdrawn from them for the rTL and other genetics analyses in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

Concept was provided by AK, SMJH, EK, and SS. Data collection was done by AK, EK, SMJH, JSW, and SS. Data analysis was done by WS, AK, RNN, SMJH, JSW, SS, MK, and JL. First draft was done by AK, SMJH, JSW, WS, EK, SS, MLJ, RNN, PK, MK, and JL. Final revision was done by AK, SMJH, JSW, EK, SS, WS, MLJ, RNN, PK, MK, and JL. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00705/full#supplementary-material

REFERENCES

Ahmadi, N., Hajsadeghi, F., Mirshkarlo, H. B., Budoff, M., Yehuda, R., and Ebrahimi, R. (2011). Post-traumatic stress disorder, coronary atherosclerosis, and mortality. *Am. J. Cardiol.* 108 (1), 29–33. doi: 10.1016/j.amjcard.2011.02.340

Allsopp, R. C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V., Futcher, A. B., et al. (1992). Telomere length predicts replicative capacity of human fibroblasts. *Proc. Natl. Acad. Sci.* 89 (21), 10114–10118. doi: 10.1073/pnas.89.21.10114

Auld, E., Lin, J., Chang, E., Byanyima, P., Ayakaka, I., Musisi, E., et al. (2016). HIV infection is associated with shortened telomere length in Ugandans with suspected tuberculosis. *PLoS One* 11 (9), e0163153. doi: 10.1371/journal.pone.0163153

Aviv, A. (2012). Genetics of leukocyte telomere length and its role in atherosclerosis. *Mutat. Res.* 730, 68–74. doi: 10.1016/j.mrfmmm.2011.05.001

Blackburn, E. H., Greider, C. W., and Szostak, J. W. (2006). Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging. *Nat. Med.* 12 (10), 1133–1138. doi: 10.1038/nm1006-1133

Cai, N., Chang, S., Li, Y., Li, Q., Hu, J., Liang, J., et al. (2015). Molecular signatures of major depression. *Curr. Biol.* 25 (9), 1146–1156. doi: 10.1016/j.cub.2015.03.008

Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res.* 37 (3), e21–e21. doi: 10.1093/nar/gkn1027

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 30, e47. doi: 10.1093/nar/30.10.e47

Cesare, A. J., and Reddel, R. R. (2010). Alternative lengthening of telomeres: models, mechanisms and implications. *Nat. Rev. Gen.* 11 (5), 319. doi: 10.1038/nrg2763

Cherkas, L. F., Hunkin, J. L., Kato, B. S., Richards, J. B., Gardner, J. P., Surdulescu, G. L., et al. (2008). The association between physical activity in leisure time and leukocyte telomere length. *Arch. Intern. Med.* 168 (2), 154–158. doi: 10.1001/archinternmed.2007.39

Colton, C. W., and Manderscheid, R. W. (2006). PEER REVIEWED: congruencies in increased mortality rates, years of potential life lost, and causes of death among public mental health clients in eight states. *Prev. Chronic Dis.* 3 (2), A42.

Cuijpers, P., and Smit, F. (2002). Excess mortality in depression: a meta-analysis of community studies. *J. Affect. Disord.* 72 (3), 227–236. doi: 10.1016/S0165-0327(01)00413-X

Damjanovic, A. K., Yang, Y., Glaser, R., Kiecolt-Glaser, J. K., Nguyen, H., Laskowski, B., et al. (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J. Immunol.* 179 (6), 4249–4254. doi: 10.4049/jimmunol.179.6.4249

Drury, S. S., Shirtcliff, E. A., Shachet, A., Phan, J., Mabile, E., Brett, Z. H., et al. (2014). Growing up or growing old? Cellular aging linked with testosterone reactivity to stress in youth. *Am. J. Med. Sci.* 348, 92–100. doi: 10.1097/MAJ.0000000000000299

Drury, S. S., Theall, K., Gleason, M. M., Smyke, A. T., De Vivo, I., Wong, J. Y. Y., et al. (2012). Telomere length and early severe social deprivation: linking early adversity and cellular aging. *Mol. Psychiatry* 17 (7), 719. doi: 10.1038/mp.2011.53

Druss, B. G., Zhao, L., Von Esenwein, S., Morrato, E. H., and Marcus, S. C. (2011). Understanding excess mortality in persons with mental illness: 17-year follow up of a nationally representative US survey. *Med. Care* 49 (6), 599–604. doi: 10.1097/MLR.0b013e31820bf86e

Epel, E. S., and Prather, A. A. (2018). Stress, telomeres, and psychopathology: toward a deeper understanding of a triad of early aging. *Annu. Rev. Clin. Psychol.* 14, 371–397. doi: 10.1146/annurev-clinpsy-032816-045054

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., et al. (2004). Accelerated telomere shortening in response to life stress. *Proc. Natl. Acad. Sci. U. S. A.* 101 (49), 17312–17315. doi: 10.1073/pnas.0407162101

Fergusson, D. M., and Mullen, P. E., (1999). *Childhood sexual abuse: an evidence based perspective*. Thousand Oaks: Sage Publications, Inc.

Gadow, K.D., and Sprafkin, J. (2013). Child and adolescent symptom inventory-5. Checkmate plus. Stony brook. Available at: https://www.checkmateplus.com/products/casi5.htm (Accessed June 6, 2019).

Gadow, K., Angelidou, K., Chernoff, M., Williams, P. L., Heston, J., Hodge, J., et al. (2012). Longitudinal study of emerging mental health concerns in youth perinatally infected with HIV and peer comparisons. *J. Dev. Behav. Pediatr.* 33 (6), 456. doi: 10.1097/DBP.0b013e31825b8482

Garcia-Rizo, C., Fernandez-Egea, E., Miller, B. J., Oliveira, C., Justicia, A., Griffith, J. K., et al. (2013). Abnormal glucose tolerance, white blood cell count, and telomere length in newly diagnosed, antidepressant-naive patients with depression. *Brain Behav. Immun.* 28, 49–53. doi: 10.1016/j.bbi.2012.11.009

Gotlib, I., LeMoult, J., Colich, N., Foland-Ross, L., Hallmayer, J., Joormann, J., et al. (2015). Telomere length and cortisol reactivity in children of depressed mothers. *Mol. Psychiatry* 20 (5), 615–620. doi: 10.1038/mp.2014.119

Haycock, P. C., Heydon, E. E., Kaptoge, S., Butterworth, A. S., Thompson, A., and Willeit, P. (2014). Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* 349, g4227. doi: 10.1136/bmj.g4227

Heuser, I. (2002). Depression, endocrinologically a syndrome of premature aging? *Maturitas* 41 (Suppl 1), S19–S23. doi: 10.1016/S0378-5122(02)00012-9

Hoen, P. W., de Jonge, P., Na, B. Y., Farzaneh-Far, R., Epel, E., Lin, J., et al. (2011). Depression and leukocyte telomere length in patients with coronary heart disease: data from the Heart and Soul Study. *Psychosom. Med.* 73, 541–547. doi: 10.1097/PSY.0b013e31821b1f6e

Kamau, J. W., Kuria, W., Mathai, M., Atwoli, L., and Kangethe, R. (2012). Psychiatric morbidity among HIV-infected children and adolescents in a resource-poor Kenyan urban community. *AIDS Care* 24 (7), 836–842. doi: 10.1080/09540121.2011.644234

Kananen, L., Surakka, I., Pirkola, S., Suvisaari, J., Lonnqvist, J., Peltonen, L., et al. (2010). Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *PLoS One* 5, e10826. doi: 10.1371/journal.pone.0010826

Kendler, K. S., Bulik, C. M., Silberg, J., Hettema, J. M., Myers, J., and Prescott, C. A. (2000). Childhood sexual abuse and adult psychiatric and substance use disorders in women: an epidemiological and cotwin control analysis. *Arch. Gen. Psychiatry* 57, 953–959. doi: 10.1001/archpsyc.57.10.953

Kendler, K. S., Karkowski, L. M., and Prescott, C. A. (1999). Causal relationship between stressful life events and the onset of major depression. *Am. J. Psychiatry* 156, 837–841. doi: 10.1176/ajp.156.6.837

Kiecolt-Glaser, J. K., Derry, H. M., and Fagundes, C. P. (2015). Inflammation: depression fans the flames and feasts on the heat. *Am. J. Psychiatry* 172 (11), 1075–1091. doi: 10.1176/appi.ajp.2015.15020152

Kim, M.H., Mazenga, A.C., Devandra, A., Ahmed, S., Kazembe, P.N., Yu, X., et al. (2014) Prevalence of depression and validation of the beck depression inventory-ii and the children's depression inventory-short amongst HIV-positive adolescents in Malawi. *J. Int. AIDS Soc.* 17 (1), 18965. doi: 10.7448/IAS.17.1.18965

Kinyanda, E., Salisbury, T.T., Levin, J., Nakasujja, N., Mpango, R.S., Abbo, C., et al. (2019). Rates, types and co-occurence of emotional and behavioural disorders among perinatally HIV-infected youth in Uganda: the CHAKA study. *Soc. Psychiatry Psychiatr. Epidemiol.* 54 (4), 415–25. doi: 10.1007/s00127-019-01675-0

Kinyanda, E., Waswa, L., Baisley, K., and Maher, D. (2011). Prevalence of severe mental distress and its correlates in a population-based study in rural South-West Uganda. *BMC Psychiatry* 11 (1), 97. doi: 10.1186/1471-244X-11-97

Knizek, B.L., Mugisha, J., Osafo, J., Kinyanda, E. (2017). Growing up HIV-positive in Uganda: "psychological immunodeficiency"? A qualitative study. *BMC Psychology* 5(1), 30. doi: 10.1186/s40359-017-0199-7

Stellenbosch University https://scholar.sun.ac.za

Le Doaré, K., Bland, R., and Newell, M. L. (2012). Neurodevelopment in children born to HIV-infected mothers by infection and treatment status. *Pediatrics* 130 (5), e1326–e1344. doi: 10.1542/peds.2012-0405

Lin, J., Epel, E., and Blackburn, E. (2012). Telomeres and lifestyle factors: roles in cellular aging. *Mutat Res* 730, 85–89. doi: 10.1016/j.mrfmmm.2011.08.003

Lindqvist, D., Epel, E. S., Mellon, S. H., Penninx, B. W., Révész, D., Verhoeven, J. E., et al. (2015). Psychiatric disorders and leukocyte telomere length: underlying mechanisms linking mental illness with cellular aging. *Neurosci. Biobehav. Rev.* 55, 333–364. doi: 10.1016/j.neubiorev.2015.05.007

Lung, F. W., Chen, N. C., and Shu, B. C. (2007). Genetic pathway of major depressive disorder in shortening telomeric length. *Psychiatr. Genet.* 17, 195–199. doi: 10.1097/YPG.0b013e32808374f6

Lwidiko, A., Kibusi, S.M., Nyundo, A., Mpondo, B.C. (2018). Association between HIV status and depressive symptoms among children and adolescents in the Southern Highlands Zone, Tanzania: a case-control study. *PloS One* 13 (2), e0193145. doi: 10.1371/journal.pone.0193145

Malan, S., Hemmings, S., Kidd, M., Martin, L., and Seedat, S. (2011). Investigation of telomere length and psychological stress in rape victims. *Depress Anxiety* 28, 1081–1085. doi: 10.1002/da.20903

Malee, K., Williams, P., Montepiedra, G., McCabe, M., Nichols, S., Sirois, P. A., et al. (2011). Medication adherence in children and adolescents with HIV infection: associations with behavioral impairment. *AIDS Patient Care STDs* 25 (3), 191–200. doi: 10.1089/apc.2010.0181

McEwen, B. S. (2003). Mood disorders and allostatic load. *Biol. Psychiatry* 54 (3), 200–207. doi: 10.1016/S0006-3223(03)00177-X

Mellins, C. A., Elkington, K. S., Leu, C.-S., Santamaria, E. K., Dolezal, C., Wiznia, A., et al. (2012). Prevalence and change in psychiatric disorders among perinatally HIV-infected and HIV-exposed youth. *AIDS care* 24 (8), 953–962. doi: 10.1080/09540121.2012.668174

Michopoulos, V., Powers, A., Gillespie, C. F., Ressler, K. J., and Jovanovic, T. (2017). Inflammation in fear- and anxiety-based disorders: PTSD, GAD, and beyond. *Neuropsychopharmacology* 42 (1), 254. doi: 10.1038/npp.2016.146

Miller, M. W., and Sadeh, N. (2014). Traumatic stress, oxidative stress and post-traumatic stress disorder: neurodegeneration and the accelerated-aging hypothesis. *Mol. Psychiatry* 19, 1156–1162. doi: 10.1038/mp.2014.111

Mitchell, C., Hobcraft, J., McLanahan, S. S., Siegel, S. R., Berg, A., Brooks-Gunn, J., et al. (2014). Social disadvantage, genetic sensitivity, and children's telomere length. *Proc. Natl Acad Sci.* 111 (16), 5944–5949. doi: 10.1073/pnas.1404293111

Moreno-Villanueva, M., Morath, J., Vanhooren, V., Elbert, T., Kolassa, S., Libert, C., et al. (2013). *N*-Glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder. *Transl. Psychiatry* 3, e320. doi: 10.1038/tp.2013.93

Mpango, R. S., Kinyanda, E., Rukundo, G. Z., Gadow, K. D., and Patel, V. (2017). Cross-cultural adaptation of the Child and Adolescent Symptom Inventory-5 (CASI-5) for use in central and South-Western Uganda: the CHAKA project. *Trop. Doct.* 47 (4), 347–354. doi: 10.1177/0049475517724688

Muezzinler, A., Zaineddin, A. K., and Brenner, H. (2013). A systematic review of leukocyte telomere length and age in adults. *Ageing Res. Rev.* 12, 509–519. doi: 10.1016/j.arr.2013.01.003

Mupambireyi, Z., Bernays, S., Bwakura-Dangarembizi, M., and Cowan, F. M. (2014). "I don't feel shy because I will be among others who are just like me…": the role of support groups for children perinatally infected with HIV in Zimbabwe. *Child. Youth Serv. Rev.* 45, 106–113. doi: 10.1016/j.childyouth.2014.03.026

Musisi, S., and Kinyanda, E. (2009). Emotional and behavioural disorders in HIV seropositive adolescents in urban Uganda. *East Afr. Med. J.* 86 (1), 16–24. doi: 10.4314/eamj.v86i1.46923

Nachman, S., Chernoff, M., Williams, P., Hodge, J., Heston, J., and Gadow, K. D. (2012). Human immunodeficiency virus disease severity, psychiatric symptoms, and functional outcomes in perinatally infected youth. *Arch. Pediatr. Adolesc. Med.* 166 (6), 528–535. doi: 10.1001/archpediatrics.2011.1785

Needham, B. L., Fernandez, J. R., Lin, J., Epel, E. S., and Blackburn, E. H. (2012). Socioeconomic status and cell aging in children. *Soc. Sci. Med.* 74, 1948–1951. doi: 10.1016/j.socscimed.2012.02.019

Needham, B. L., Mezuk, B., Bareis, N., Lin, J., Blackburn, E. H., and Epel, E. S. (2015). Depression, anxiety and telomere length in young adults: evidence from the National Health and Nutrition Examination Survey. *Mol. Psychiatry* 20 (4), 520. doi: 10.1038/mp.2014.89

Njajou, O. T., Cawthon, R. M., Damcott, C. M., Wu, S.-H., Ott, S., Garant, M. J., et al. (2007). Telomere length is paternally inherited and is associated with parental lifespan. *Proc. Natl Acad Sci.* 104 (29), 12135–12139. doi: 10.1073/pnas.0702703104

O'Donovan, A., Epel, E., Lin, J., Wolkowitz, O., Cohen, B., Maguen, S., et al. (2011). Childhood trauma associated with short leukocyte telomere length in posttraumatic stress disorder. *Biol. Psychiatry* 70, 465–471. doi: 10.1016/j.biopsych.2011.01.035

Oeseburg, H., de Boer, R. A., van Gilst, W. H., and van der Harst, P. (2010). Telomere biology in healthy aging and disease. *Pflügers Archiv-Eur. J. Physiol.* 459 (2), 259–268. doi: 10.1007/s00424-009-0728-1

Okereke, O. I., Prescott, J., Wong, J. Y., Han, J., Rexrode, K. M., and De Vivo, I. (2012). High phobic anxiety is related to lower leukocyte telomere length in women. *PLoS One* 7, e40516. doi: 10.1371/journal.pone.0040516

Regier, D. A., Kuhl, E. A., and Kupfer, D. J. (2013). The DSM-5: classification and criteria changes. *World Psychiatry* 12 (2), 92–98. doi: 10.1002/wps.20050

Ridout, S. J., Ridout, K. K., Kao, H. T., Carpenter, L. L., Philip, N. S., Tyrka, A. R., et al. (2015). Telomeres, early-life stress and mental illness. *Adv. Psychosom. Med.* 34, 92–108. doi: 10.1159/000369088

Schaakxs, R., Verhoeven, J. E., Oude Voshaar, R. C., Comijs, H. C., and Penninx, B. W. (2015). Leukocyte telomere length and late-life depression. *Am. J. Geriatr. Psychiatry* 23, 423–432. doi: 10.1016/j.jagp.2014.06.003

Shalev, I., Entringer, S., Wadhwa, P. D., Wolkowitz, O. M., Puterman, E., Lin, J., et al. (2013). Stress and telomere biology: a lifespan perspective. *Psychoneuroendocrinology* 38 (9), 1835–1842. doi: 10.1016/j.psyneuen.2013.03.010

Shalev, I., Moffitt, T. E., Braithwaite, A. W., Danese, A., Fleming, N. I., Goldman-Mellor, S., et al. (2014). Internalizing disorders and leukocyte telomere erosion: a prospective study of depression, generalized anxiety disorder and post-traumatic stress disorder. *Mol. Psychiatry* 19 (11), 1163–1170. doi: 10.1038/mp.2013.183

Shiels, P. G., McGlynn, L. M., MacIntyre, A., Johnson, P. C., Batty, G. D., Burns, H., et al. (2011). Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *PloS One* 6 (7), e22521. doi: 10.1371/journal.pone.0022521

Simon, N. M., Smoller, J. W., McNamara, K. L., Maser, R. S., Zalta, A. K., Pollack, M. H., et al. (2006). Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biol. Psychiatry* 60 (5), 432–435. doi: 10.1016/j.biopsych.2006.02.004

Simon, N. M., Walton, Z. E., Bui, E., Prescott, J., Hoge, E., Keshaviah, A., et al. (2015). Telomere length and telomerase in a well-characterized sample of individuals with major depressive disorder compared to controls. *Psychoneuroendocrinology* 58, 9–22. doi: 10.1016/j.psyneuen.2015.04.004

Tandon, M., Si, X., and Luby, J. (2011). Preschool onset attention-deficit/hyperactivity disorder: course and predictors of stability over 24 months. *J. Child. Adolesc. Psychopharmacol.* 21 (4), 321–330. doi: 10.1089/cap.2010.0045

Teyssier, J. R., Chauvet-Gelinier, J. C., Ragot, S., and Bonin, B. (2012). Up-regulation of leucocytes genes implicated in telomere dysfunction and cellular senescence correlates with depression and anxiety severity scores. *PLoS One* 7, e49677. doi: 10.1371/journal.pone.0049677

Theall, K. P., Brett, Z. H., Shirtcliff, E. A., Dunn, E. C., and Drury, S. S. (2013). Neighborhood disorder and telomeres: connecting children's exposure to community level stress and cellular response. *Soc. Sci. Med.* 85, 50–58. doi: 10.1016/j.socscimed.2013.02.030

Turygin, N., Matson, J. L., Beighley, J., and Adams, H. (2013). The effect of DSM-5 criteria on the developmental quotient in toddlers diagnosed with autism spectrum disorder. *Dev. Neurorehabil.* 16 (1), 38–43. doi: 10.3109/17518423.2012.712065

UNAIDS. (2010). UNAIDS Report on the Global AIDS Epidemic. Available from: https://www.unaids.org/globalreport/Global report.htm. (Accessed May 16, 2019).

UNAIDS. (2018). HIV & AIDS statistics—2018 fact sheet. Accessed on 27th October 2018. http://www.unaids.org/en/resources/fact-sheet.

UPHIA (2016–2017). Uganda population based HIV impact assessment 2017 [21 June 2018]. Available from: http://www.afro.who.int/sites/default/files/2017-08/UPHIA%20Uganda%20factsheet.pdf. (Accessed 02 June 2019).

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Van Rie, A., Harrington, P. R., Dow, A., and Robertson, K. (2007). Neurologic and neurodevelopmental manifestations of pediatric HIV/AIDS: a global perspective. *Eur. J. Paediatr Neurol.* 11 (1), 1–9. doi: 10.1016/j.ejpn.2006.10.006

Varni, S. E., Miller, C. T., McCuin, T., and Solomon, S. (2012). Disengagement and engagement coping with HIV/AIDS stigma and psychological well-being of people with HIV/AIDS. *J. Soc. Clin. Psychol.* 31 (2), 123–150. doi: 10.1521/jscp.2012.31.2.123

Vasa-Nicotera, M., Brouilette, S., Mangino, M., Thompson, J. R., Braund, P., Clemitson, J.-R., et al. (2005). Mapping of a major locus that determines telomere length in humans. *Am. J. Hum. Gen.* 76 (1), 147–151. doi: 10.1086/426734

Verhoeven, J. E., Revesz, D., Epel, E. S., Lin, J., Wolkowitz, O. M., and Penninx, B. W. (2014). Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. *Mol. Psychiatry* 19, 895–901. doi: 10.1038/mp.2013.151

Verhoeven, J. E., Révész, D., van Oppen, P., Epel, E. S., Wolkowitz, O. M., and Penninx, B. W. (2015). Anxiety disorders and accelerated cellular ageing. *Br. J. Psychiatry* 206 (5), 371–378. doi: 10.1192/bjp.bp.114.151027

Walkup, J., Akincigil, A., Bilder, S., Rosato, N. S., and Crystal, S. (2009). Psychiatric diagnosis and antiretroviral adherence among adolescent Medicaid beneficiaries diagnosed with human immunodeficiency virus/acquired immunodeficiency syndrome. *J. Nerv. Ment. Dis.* 197 (5), 354–361. doi: 10.1097/NMD.0b013e3181a208af

Wang, C., and Meier, U. T. (2004). Architecture and assembly of mammalian H/ACA small nucleolar and telomerase ribonucleoproteins. *EMBO J.* 23 (8), 1857–1867. doi: 10.1038/sj.emboj.7600181

Watson, J. D. (1972). Origin of concatemeric T7 DNA. *Nature (London) New Biol.* 239, 197–201. doi: 10.1038/newbio239197a0

Wikgren, M., Karlsson, T., Lind, J., Nilbrink, T., Hultdin, J., Sleegers, K., et al. (2012). Longer leukocyte telomere length is associated with smaller hippocampal volume among non-demented APOE epsilon3/epsilon3 subjects. *PLoS One* 7, e34292. doi: 10.1371/journal.pone.0034292

Wolkowitz, O. M., Mellon, S. H., Epel, E. S., Lin, J., Reus, V. I., Rosser, R., et al. (2012). Resting leukocyte TA is elevated in major depression and predicts treatment response. *Mol. Psychiatry* 17, 164–172. doi: 10.1038/mp.2010.133

Wolkowitz, O. M., Mellon, S. H., Epel, E. S., Lin, J., Dhabhar, F. S., Su, Y., et al. (2011a). Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress-preliminary findings. *PLoS One* 6 (3), e17837. doi: 10.1371/journal.pone.0017837

Wolkowitz, O. M., Reus, V. I., and Mellon, S. H. (2011b). Of sound mind and body: depression, disease, and accelerated aging. *Dialogues Clin. Neurosci.* 13 (1), 25–39

Wong, J. Y., De Vivo, I., Lin, X., Fang, S. C., and Christiani, D. C. (2014). The relationship between inflammatory biomarkers and telomere length in an occupational prospective cohort study. *PLoS One* 9 (1), e87348. doi: 10.1371/journal.pone.0087348

Zhang, L., Hu, X. Z., Benedek, D. M., Fullerton, C. S., Forsten, R. D., Naifeh, J. A., et al. (2014). The interaction between stressful life events and leukocyte telomere length is associated with PTSD. *Mol. Psychiatry* 19, 855–856. doi: 10.1038/mp.2013.141

Zhao, J., Miao, K., Wang, H., Ding, H., and Wang, D. W. (2013). Association between telomere length and type 2 diabetes mellitus: a meta-analysis. *PLoS One* 8 (11), e79993. doi: 10.1371/journal.pone.0079993

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ADDENDUM C

Genetic loci identified by Wray *et al.* (2018)

Arginine-glutamic acid dipeptide repeats *(RERE),* Solute carrier family 45 member 1 (*SLC45A1),* Neuronal Growth Regulator 1 (*NEGR1),* Long Intergenic Non-Protein Coding RNA 1360 (*LINC01360),* DENN Domain Containing 1B (*DENND1B*), VRK Serine/Threonine Kinase 2 (*VRK2*), Long Intergenic Non-Protein Coding RNA 1876 (*LINC01876),* Nuclear Receptor Subfamily 4 Group A Member 2 (*NR4A2),* Glycerol-3-Phosphate Dehydrogenase 2 (*GPD2),* Testis And Ovary Specific PAZ Domain Containing 1 (*TOPAZ1),* T Cell Activation Inhibitor, Mitochondrial (*TCAIM*), Zinc Finger Protein 445 *(ZNF445),* Arginine And Serine Rich Coiled-Coil 1 (*RSRC1),* Uncharacterized gene *(LOC100996447),* Myeloid Leukemia Factor 1 (*MLF1),* Solute Carrier Family 30 Member 9 *(SLC30A9),* Long Intergenic Non-Protein Coding RNA 682 *(LINC00682),* DDB1 And CUL4 Associated Factor 4 Like 1 *(DCAF4L1),* Long Intergenic Non-Protein Coding RNA 461 (*LINC00461),* Myocyte-specific enhancer factor 2C *(MEF2C),* Uncharacterized *(LOC1019),* Teneurin Transmembrane Protein 2 (*TENM2*), Extended MHC, F-Box And Leucine Rich Repeat Protein 4 (*FBXL4),* Uncharacterized protein C6orf168 *(C6orf168),* TMEM106B transmembrane protein 106B *(TMEM106B*), Von Willebrand Factor D And EGF Domains (*VWDE),* Pumilio RNA Binding Family Member 3 *(PUM3),* Long Intergenic Non-Protein Coding RNA 1231 *(LINC01231),* Astrotactin 2 *(ASTN2*), DENN Domain Containing 1A *(DENND1A),* LIM Homeobox 2 *(LHX2),* Sortilin Related VPS10 Domain Containing Receptor 3 *(SORCS3),* Uncharacterized LOC440034 *(DKFZp686K1684*), PAX6 Upstream Antisense RNA *(PAUPAR),* Elongator Acetyltransferase Complex Subunit 4 *(ELP4),* Paired box protein Pax-6 (*PAX6*), SRY-Box 5 (*SOX5*), Ecto-NOX Disulfide-Thiol Exchanger 1 *(ENOX1),* Laccase Domain Containing 1 *(LACC1),* Coiled-Coil Domain Containing 122 *(CCDC122),* Olfactomedin 4 *(OLFM4*), Long Intergenic Non-Protein Coding RNA 1065 *(LINC01065),* Leucine Rich Repeat And Fibronectin Type III Domain Containing 5 *(LRFN5),* Spectrin Repeat Containing Nuclear Envelope Protein 2 (*SYNE2),* MicroRNA 548h-1 *(MIR548H1),* Estrogen Receptor 2 *(ESR2).* Dihydrolipoamide S-Succinyltransferase *(DLST), PROX2* prospero homeobox 2 *(PROX2),* Ribosomal Protein S6 Kinase Like 1 (*RPS6KL1*), BCL2 Associated Athanogene 5 (*BAG5),* Apoptogenic, mitochondrial 1 *(APOPT1),* RNA Binding Fox-1 Homolog 1 *(RBFOX1),* Shisa Family Member 9 *(SHISA9),* Calcineurin Like Phosphoesterase Domain Containing 1 (*CPPED1*), Polyamine Modulated Factor 1 Binding Protein 1 *(PMFBP1),* DEAH-Box Helicase 38 *(DHX38),* Crystallin Beta A1 (*CRYBA1),* Myosin XVIIIA *(MYO18A),* Nuclear

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FMR1 Interacting Protein 2 (*NUFIP2)*, MIR924 host gene (*MIR924HG)*, Deleted in Colorectal Cancinona *(DCC)*, MicroRNA 4528 *(MIR4528)*, Member RAS Oncogene Family *(RAB27B)*, Coiled-Coil Domain Containing 68 (*CCDC68*), transcription factor 4 *(TCF4)*, MicroRNA 4529 (*MIR4529)*, L3MBTL Histone Methyl-Lysine Binding Protein 2 (*L3MBTL2*), *EP300* Antisense RNA 1*(EP300-AS1)*, Chondroadherin Like *(CHADL)*

Arginine-glutamic acid dipeptide repeats (*RERE*), Solute carrier family 45 member 1 (*SLC45A1*), Neuronal Growth Regulator 1 (*NEGR1*), Long Intergenic Non-Protein Coding RNA 1360 (*LINC01360*), DENN Domain Containing 1B (*DENND1B*), VRK Serine/Threonine Kinase 2 (*VRK2*), Long Intergenic Non-Protein Coding RNA 1876 (*LINC01876)*, Nuclear Receptor Subfamily 4 Group A Member 2 (*NR4A2*), Glycerol-3-Phosphate Dehydrogenase 2 (*GPD2*), Testis And Ovary Specific PAZ Domain Containing 1 (*TOPAZ1)*, T Cell Activation Inhibitor, Mitochondrial (*TCAIM*), Zinc Finger Protein 445 (*ZNF445*), Arginine And Serine Rich Coiled-Coil 1 (*RSRC1*), Uncharacterized gene (*LOC100996447*), Myeloid Leukemia Factor 1 (*MLF1*), Solute Carrier Family 30 Member 9 (*SLC30A9*), Long Intergenic Non-Protein Coding RNA 682 (*LINC00682*), DDB1 And CUL4 Associated Factor 4 Like 1 (*DCAF4L1*), Long Intergenic Non-Protein Coding RNA 461 (*LINC00461*), Myocyte-specific enhancer factor 2C (*MEF2C*), Uncharacterized (*LOC1019*), Teneurin Transmembrane Protein 2 (*TENM2*), Extended MHC, F-Box And Leucine Rich Repeat Protein 4 (*FBXL4*), Uncharacterized protein C6orf168 (*C6orf168*), TMEM106B transmembrane protein 106B (*TMEM106B*), Von Willebrand Factor D And EGF Domains (*VWDE*), Pumilio RNA Binding Family Member 3 (*PUM3*), Long Intergenic Non-Protein Coding RNA 1231 (*LINC01231*), Astrotactin 2 (*ASTN2*), DENN Domain Containing 1A (*DENND1A*), LIM Homeobox 2 (*LHX2*), Sortilin Related VPS10 Domain Containing Receptor 3 (*SORCS3*), Uncharacterized LOC440034 (*DKFZp686K1684*), PAX6 Upstream Antisense RNA (*PAUPAR*), Elongator Acetyltransferase Complex Subunit 4 (*ELP4*), Paired box protein Pax-6 (*PAX6*), SRY-Box 5 (*SOX5*), Ecto-NOX Disulfide-Thiol Exchanger 1 (*ENOX1*), Laccase Domain Containing 1 (*LACC1*), Coiled-Coil Domain Containing 122 (*CCDC122*), Olfactomedin 4 (*OLFM4*), Long Intergenic Non-Protein Coding RNA 1065 (*LINC01065*), Leucine Rich Repeat And Fibronectin Type III Domain Containing 5 (*LRFN5*), Spectrin Repeat Containing Nuclear Envelope Protein 2 (*SYNE2*), MicroRNA 548h-1 (MIR548H1), Estrogen Receptor 2 (ESR2). Dihydrolipoamide S-Succinyltransferase (DLST), PROX2 prospero homeobox 2 (PROX2), Ribosomal Protein S6 Kinase Like 1 (RPS6KL1), BCL2 Associated Athanogene 5 (BAG5), Apoptogenic, mitochondrial 1 (APOPT1), RNA Binding Fox-1 Homolog 1 (RBFOX1), Shisa Family Member 9 (SHISA9), Calcineurin Like Phosphoesterase Domain Containing 1

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(CPPED1), Polyamine Modulated Factor 1 Binding Protein 1 (PMFBP1), DEAH-Box Helicase 38 (DHX38), Crystallin Beta A1 (CRYBA1), Myosin XVIIIA (MYO18A), Nuclear FMR1 Interacting Protein 2 (NUFIP2), MIR924 host gene (MIR924HG), Deleted in Colorectal Cancinona (DCC), MicroRNA 4528 (MIR4528), Member RAS Oncogene Family (RAB27B), Coiled-Coil Domain Containing 68 (CCDC68), transcription factor 4 (TCF4), MicroRNA 4529 (MIR4529), L3MBTL Histone Methyl-Lysine Binding Protein 2 (*L3MBTL2*), EP300 Antisense RNA 1(*EP300-AS1*), Chondroadherin Like (*CHADL*).

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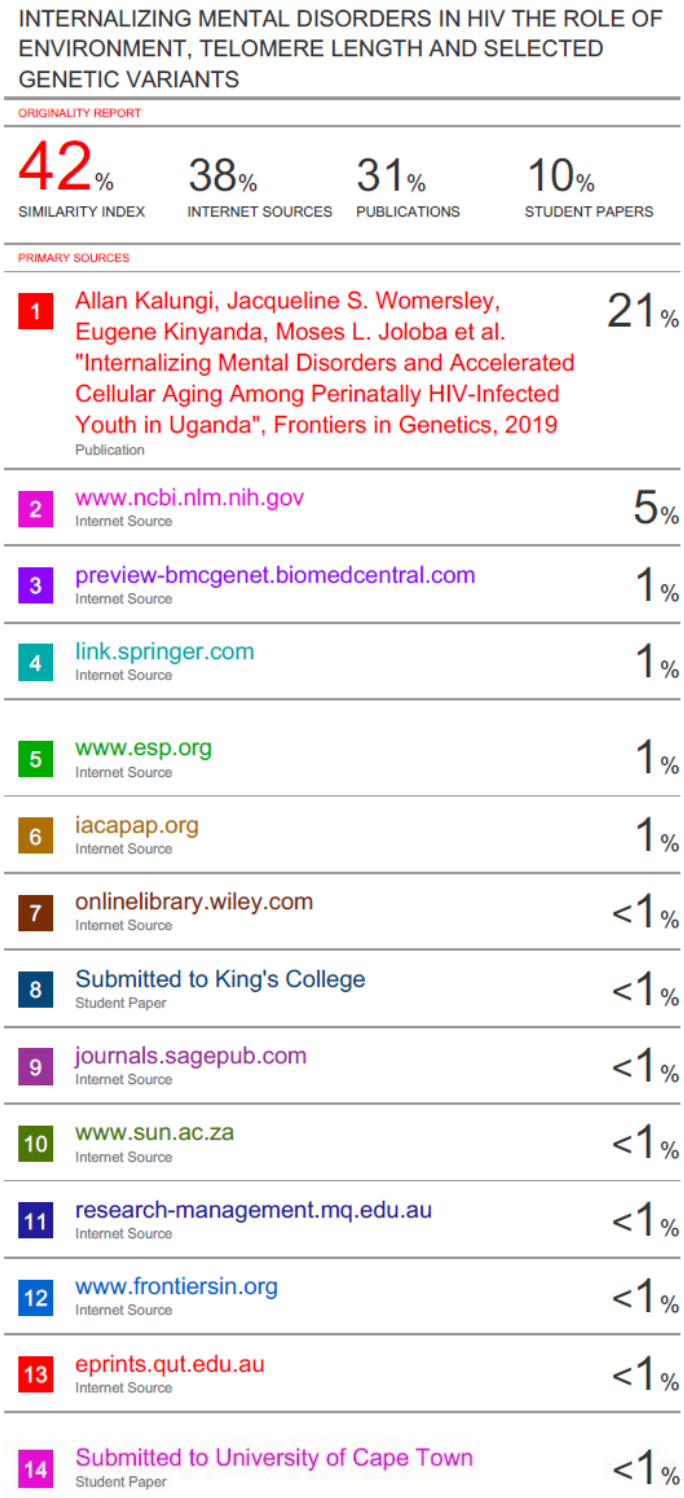
ADDENDUM D

The turnitin report for this PhD dissertation.



INTERNALIZING MENTAL DISORDERS IN HIV THE ROLE OF ENVIRONMENT, TELOMERE LENGTH AND SELECTED GENETIC VARIANTS

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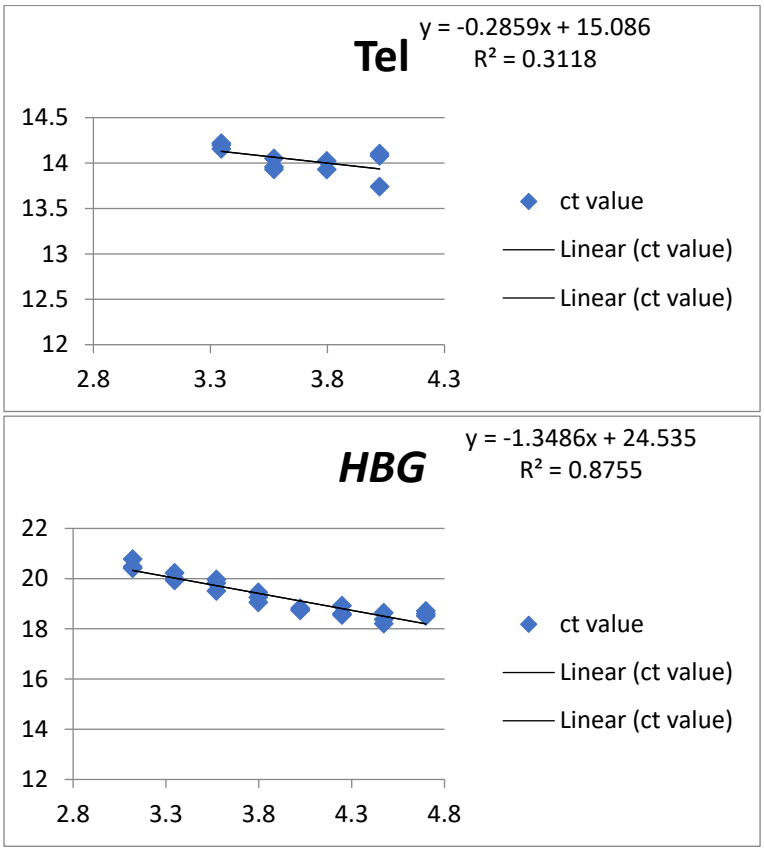
ADDENDUM E

Graphs for plot of Ct values against the log of the sample concentration for both TL and *HBG*, as well as the dissociation curve for each plate

Plate 1



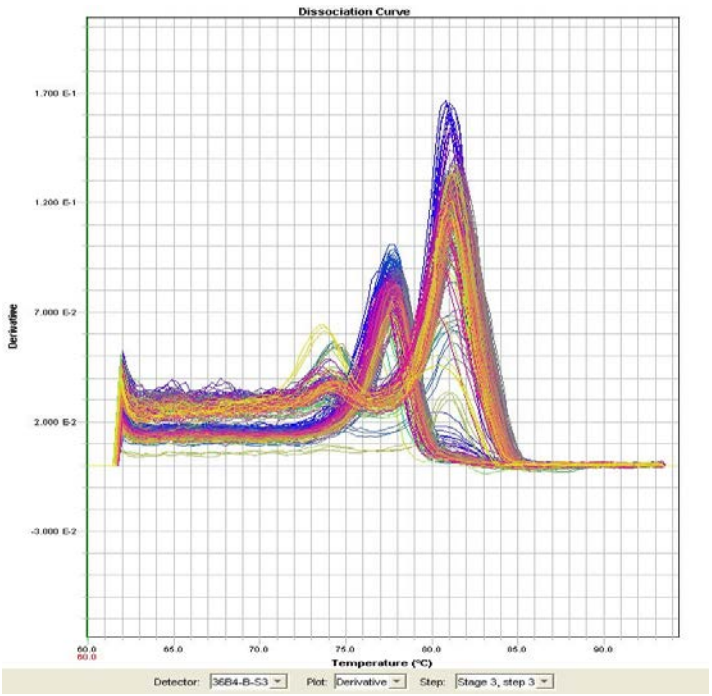


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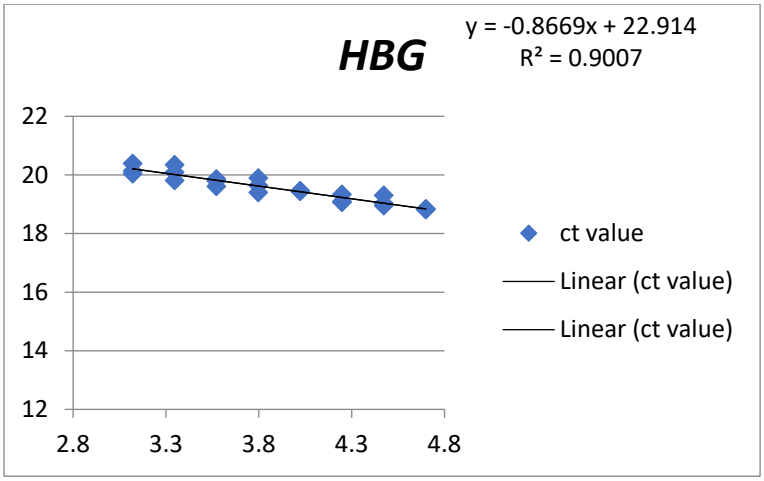
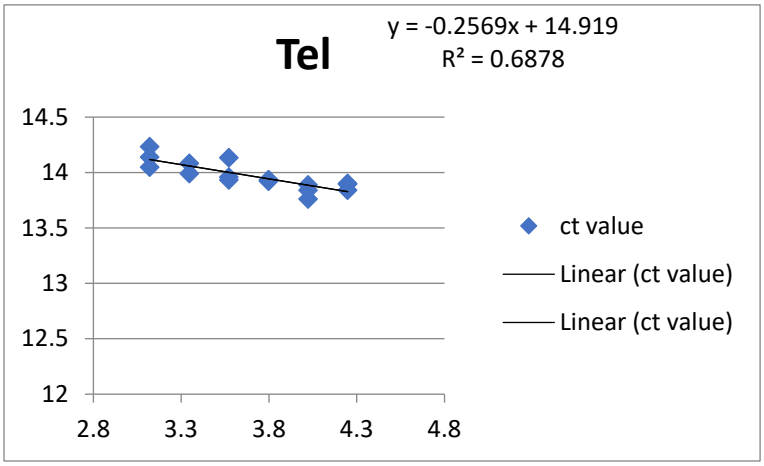
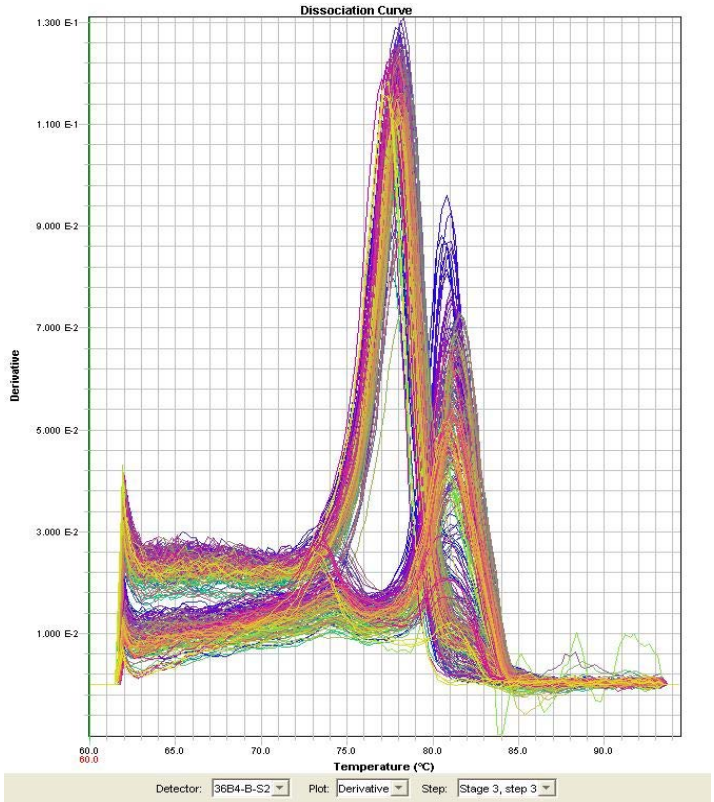
Plate 2







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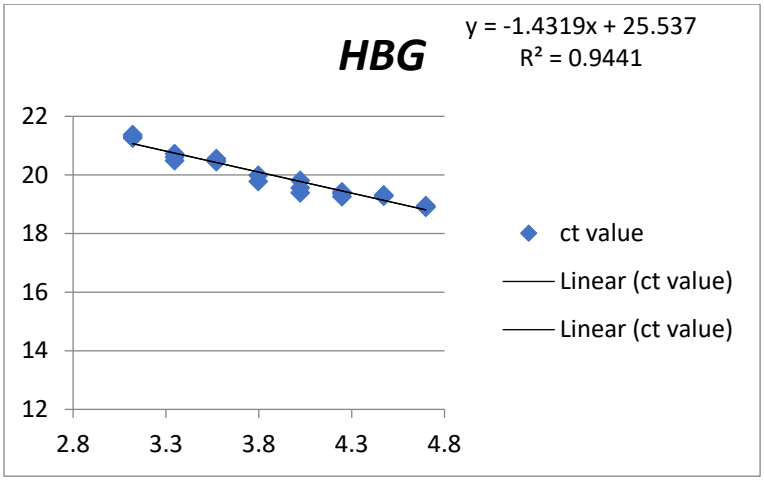

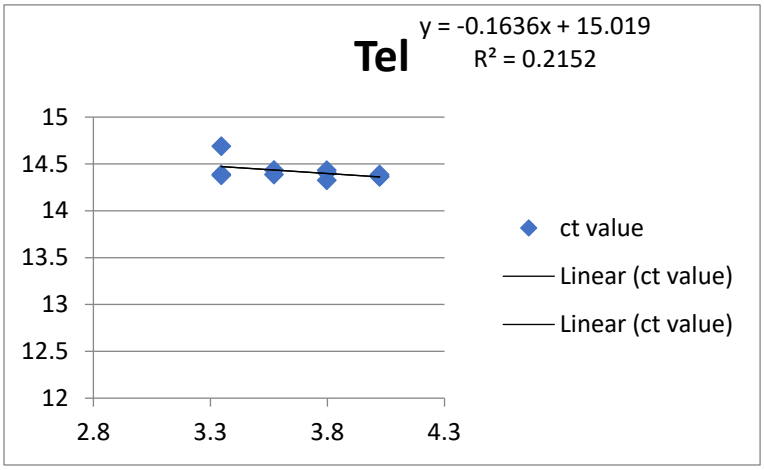

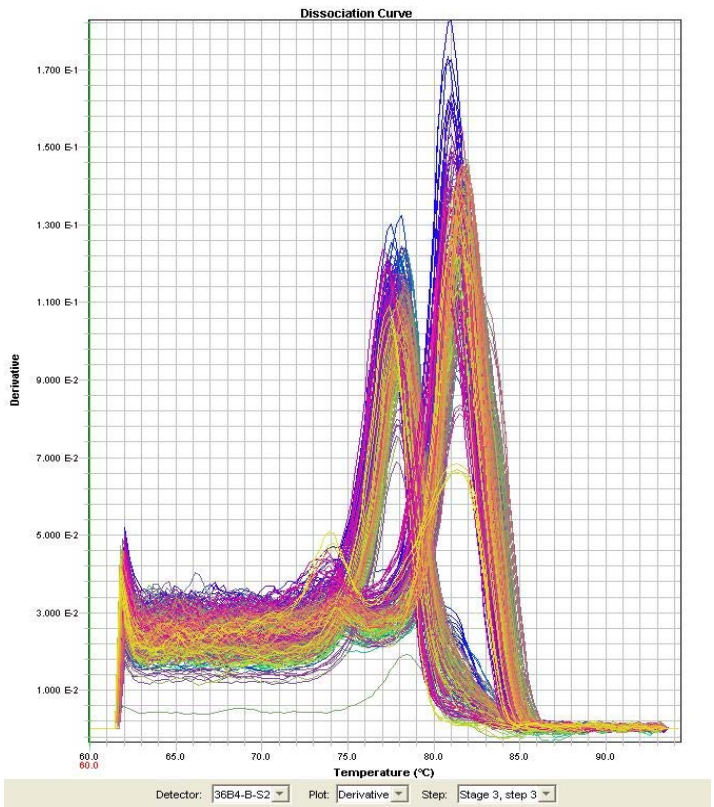

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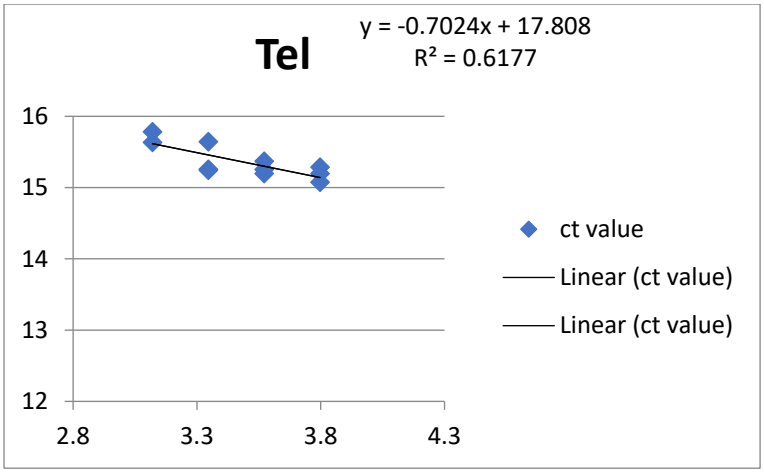

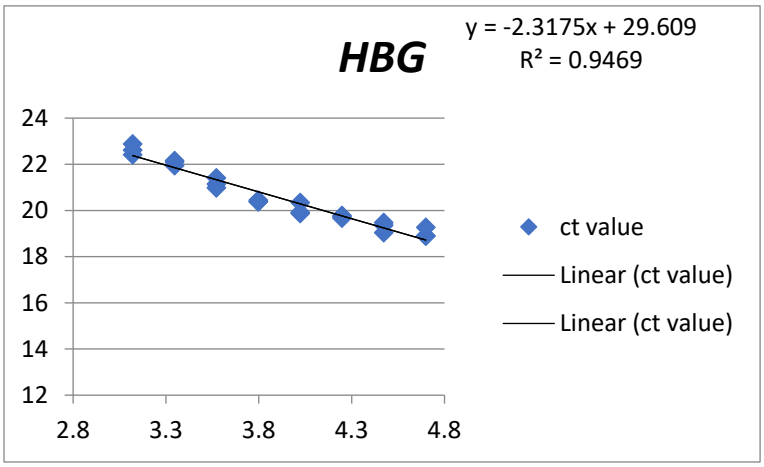

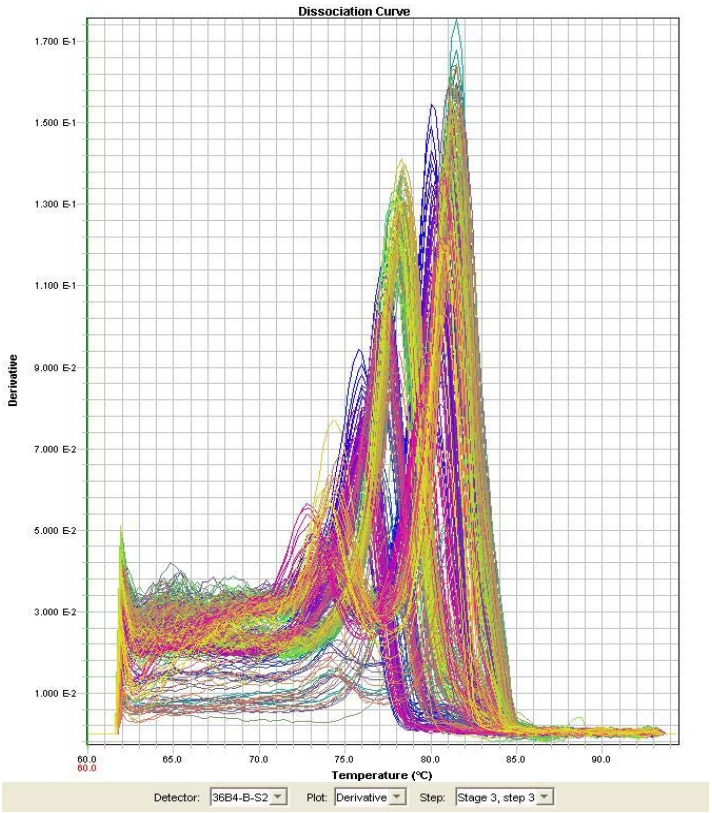

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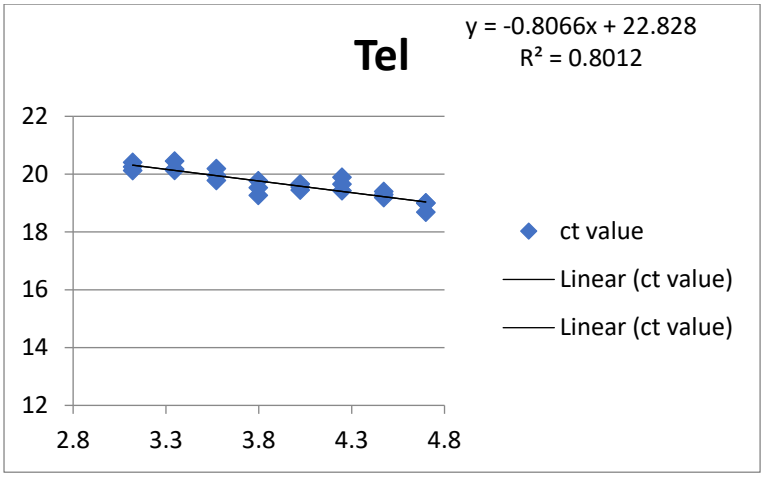

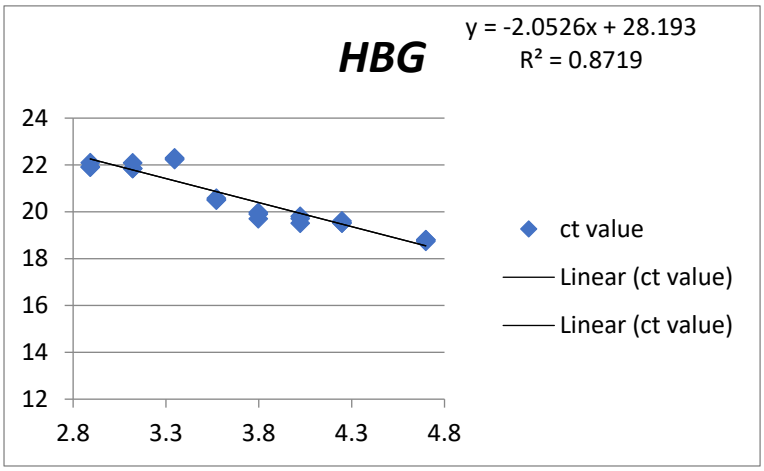

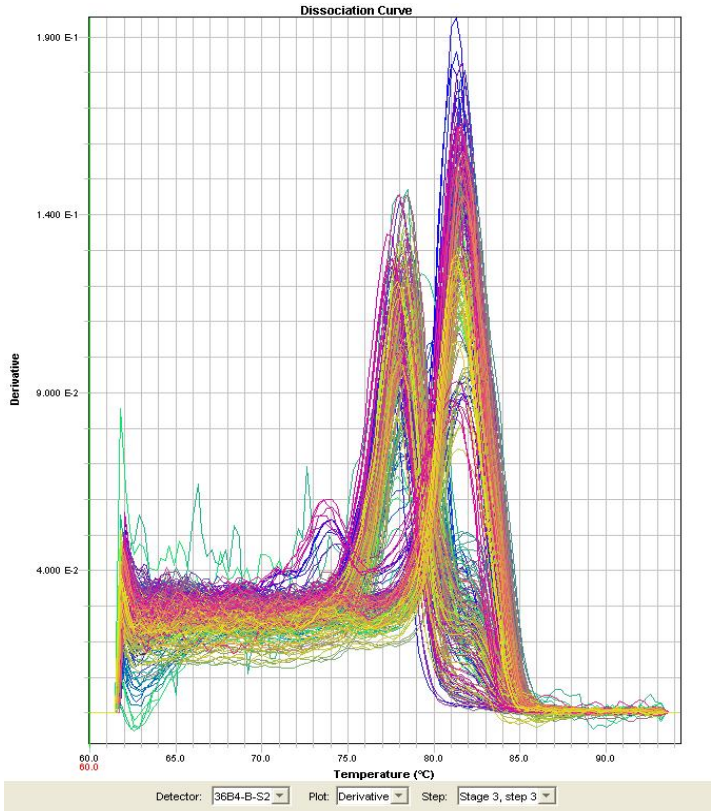

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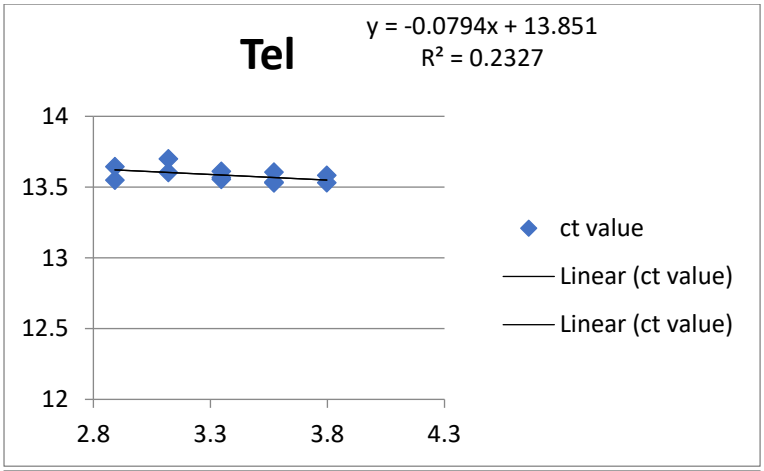
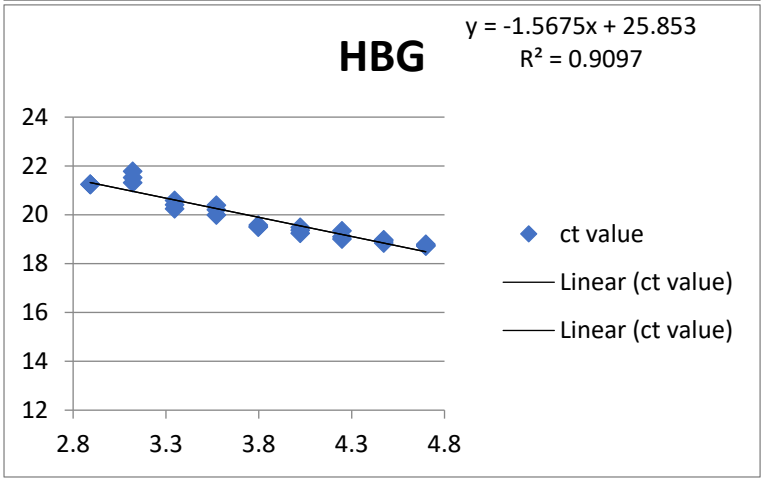
Plate 6





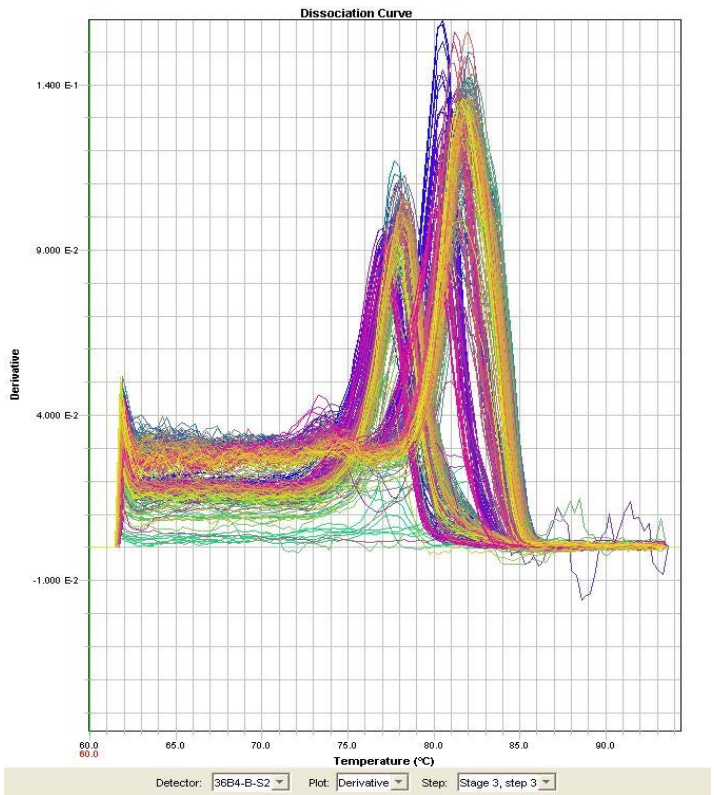


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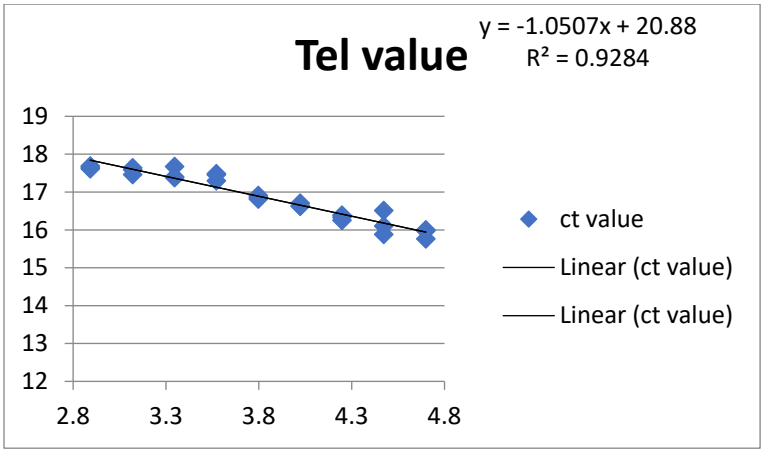
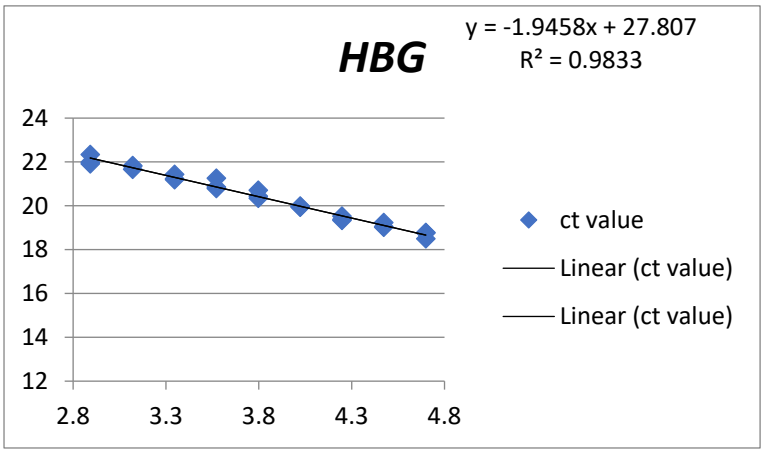
Plate 7





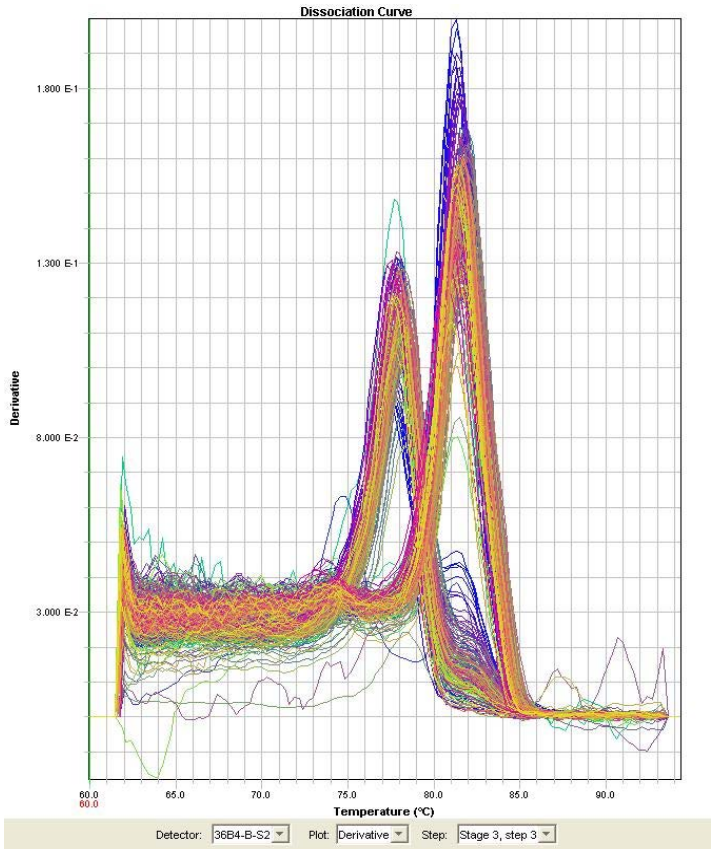


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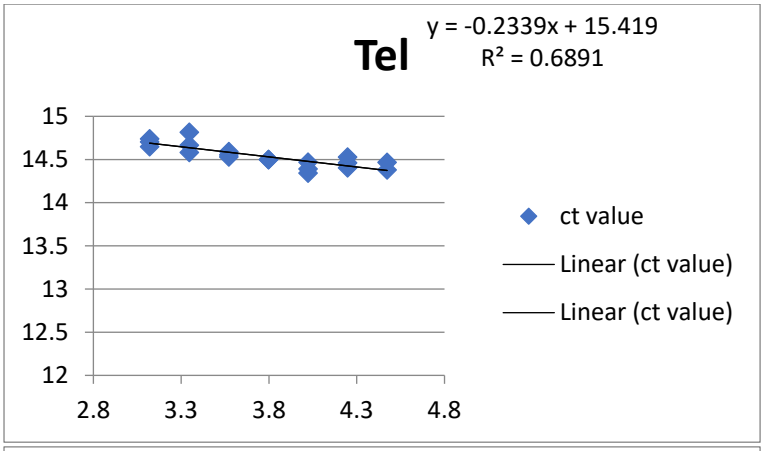
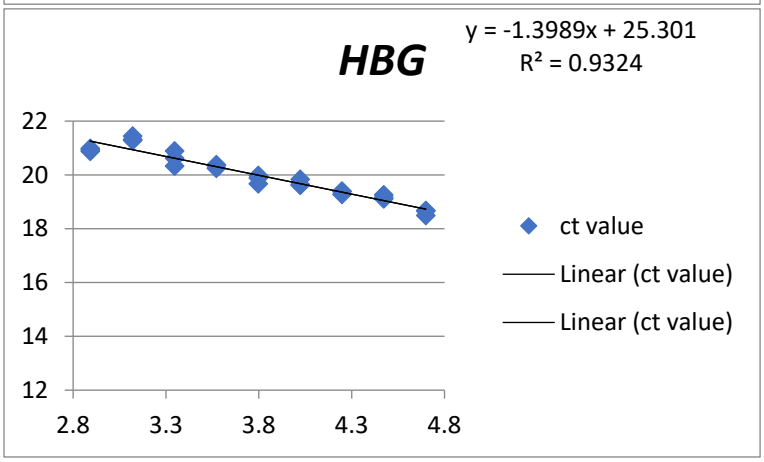
Plate 8





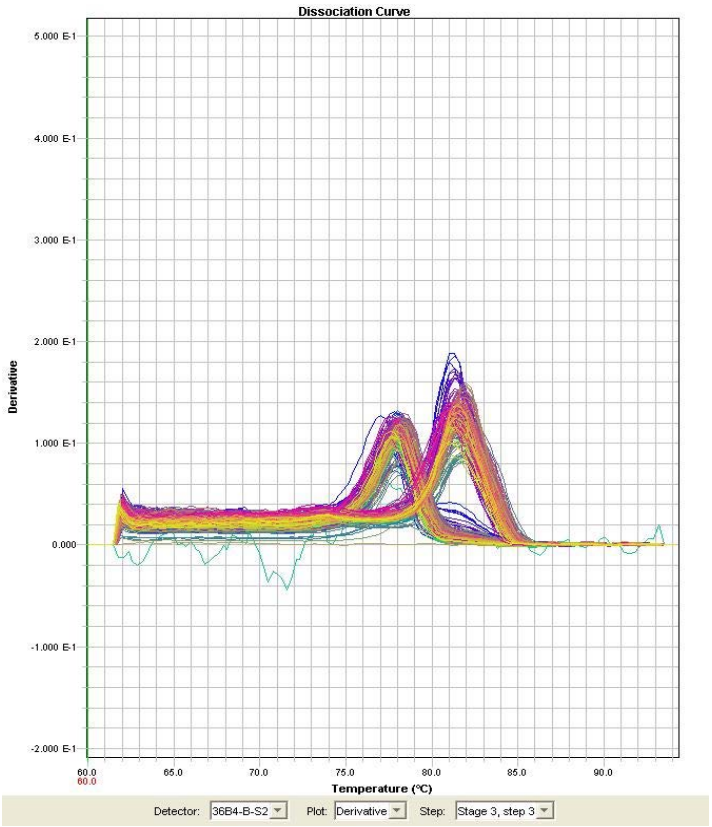


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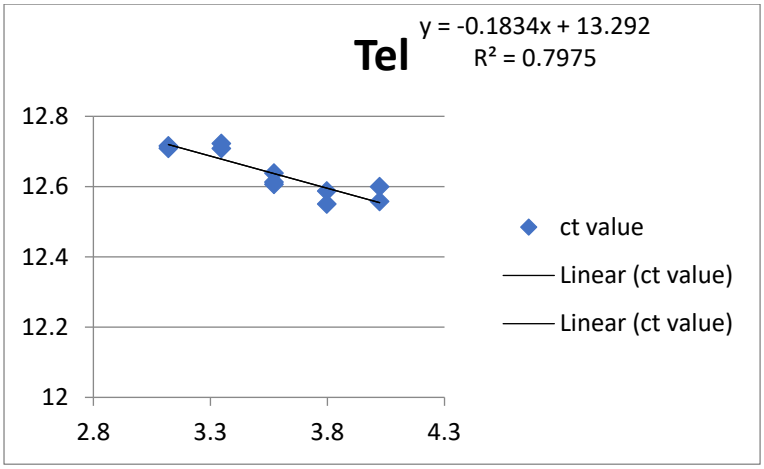
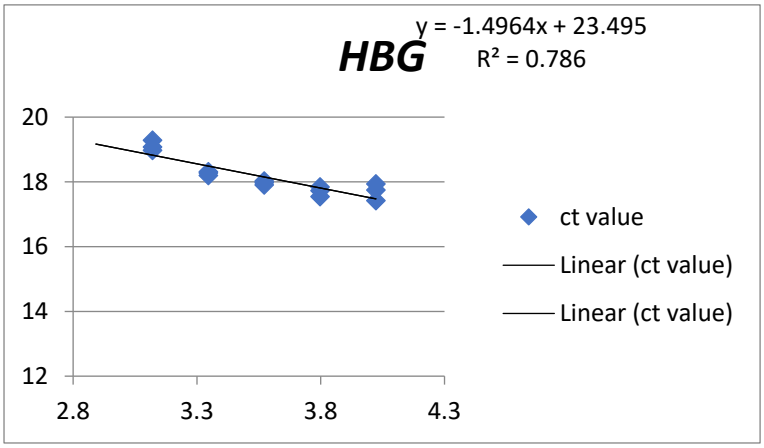
Plate 9





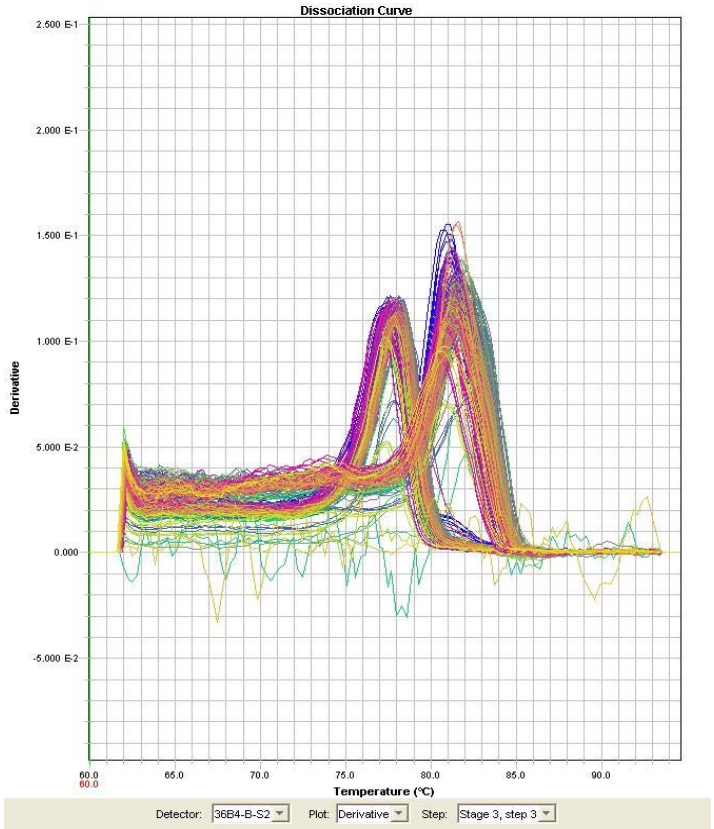


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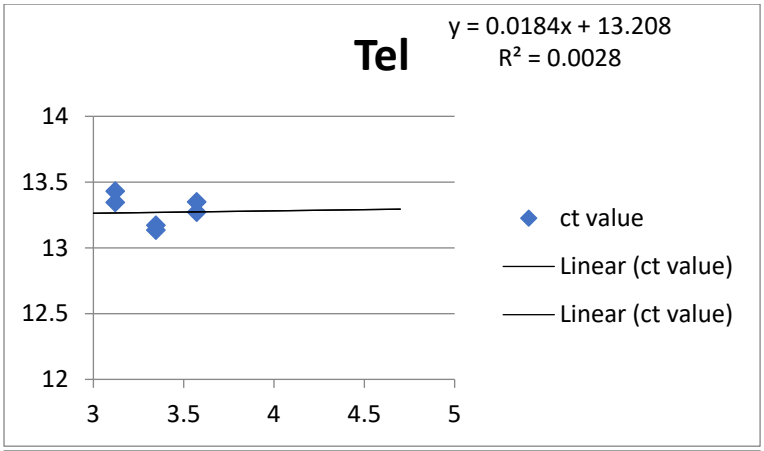
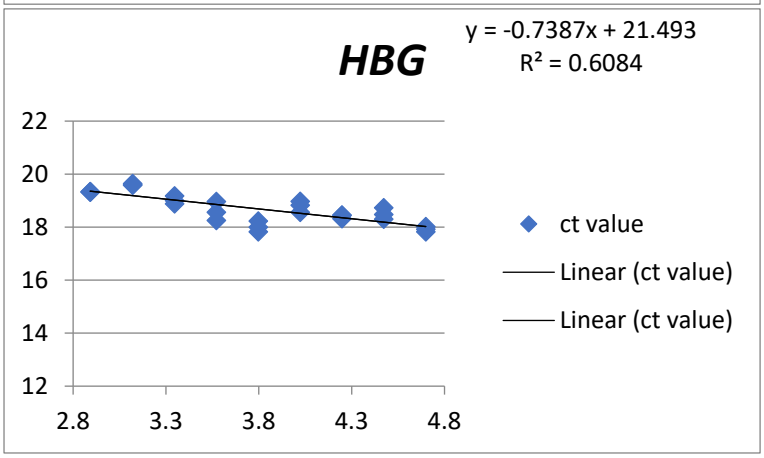
Plate 10





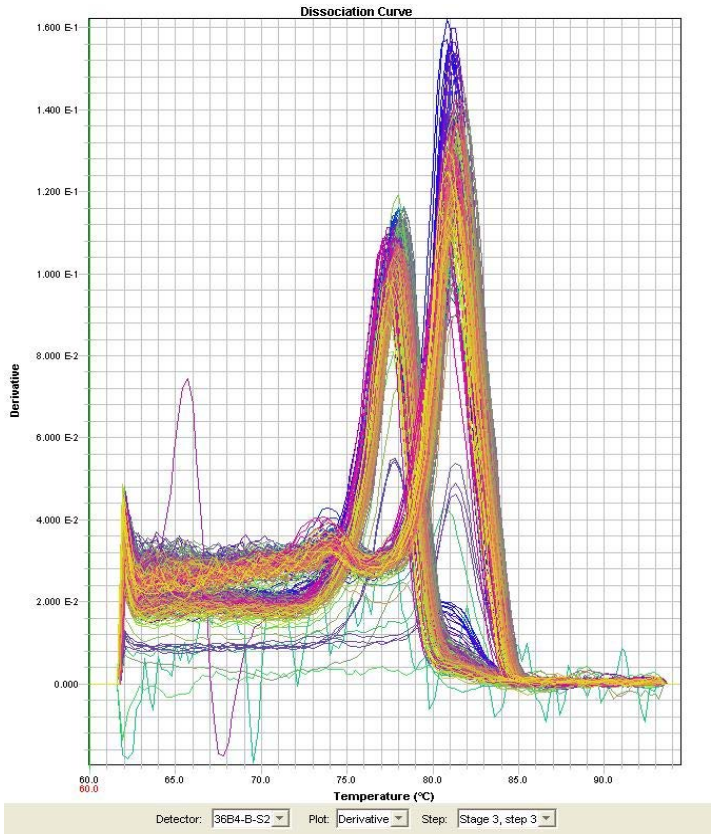


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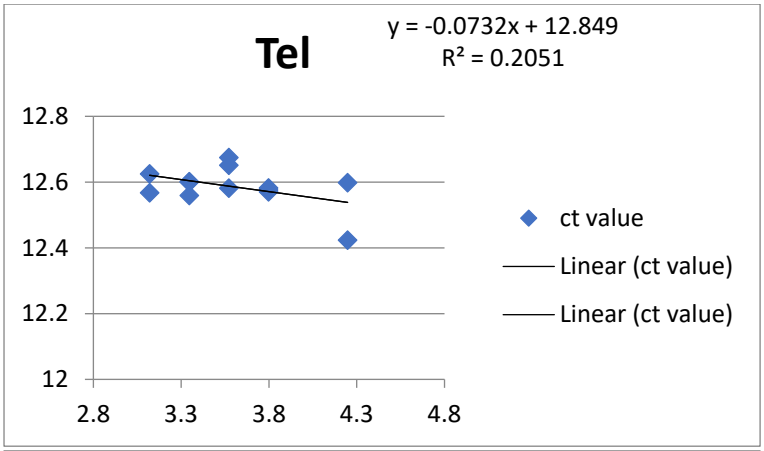
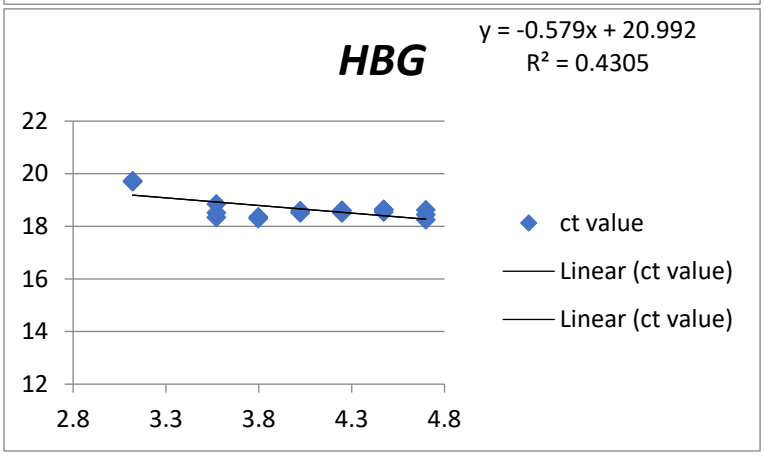
Plate 11





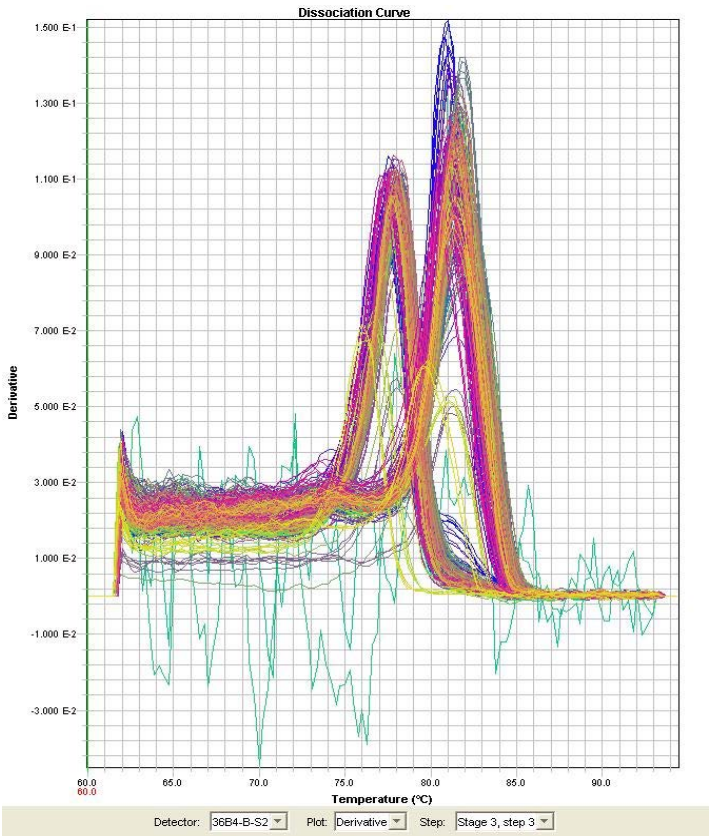


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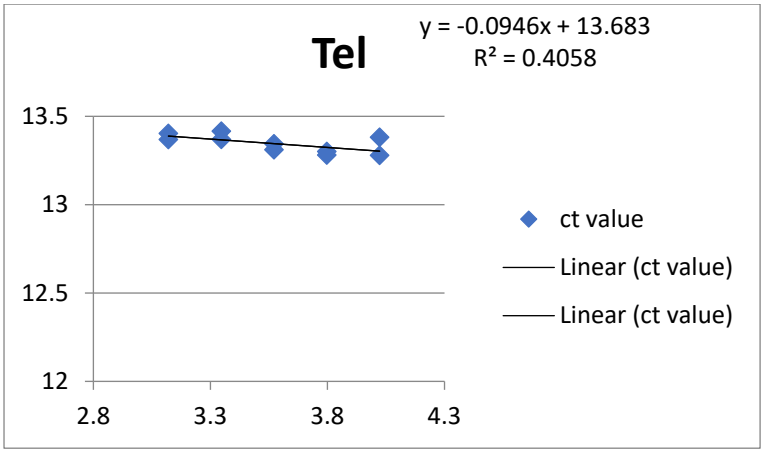
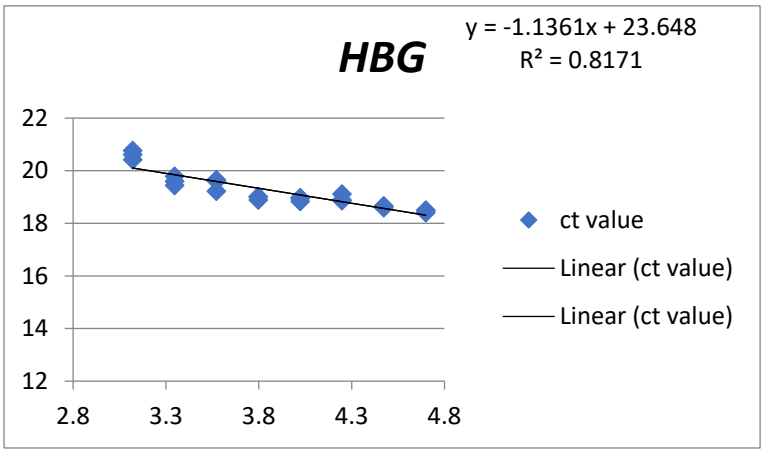
Plate 12





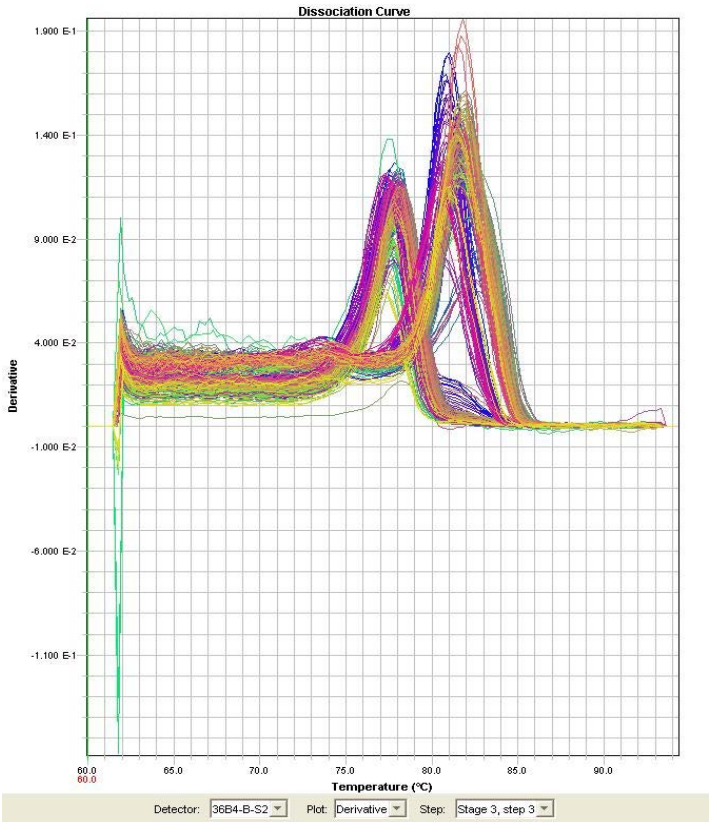


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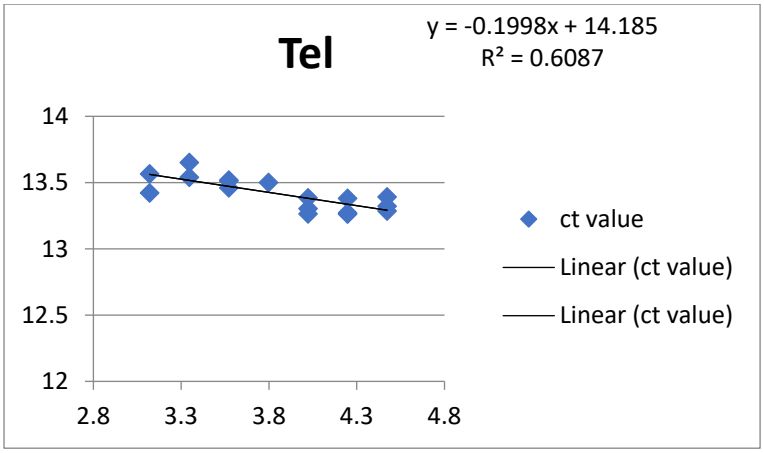
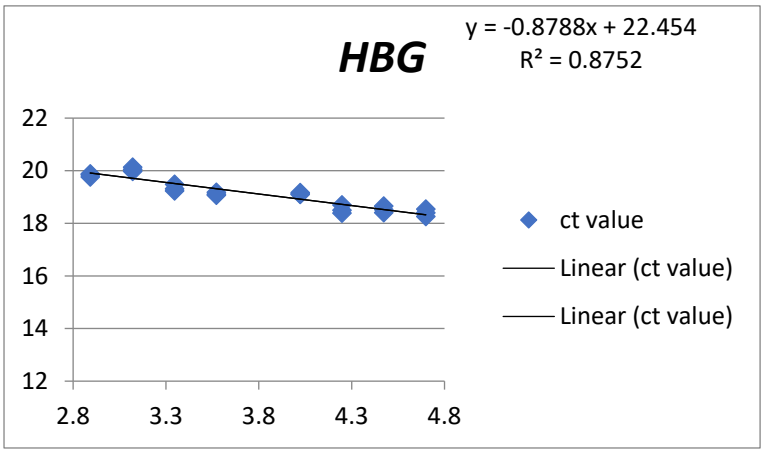
Plate 13





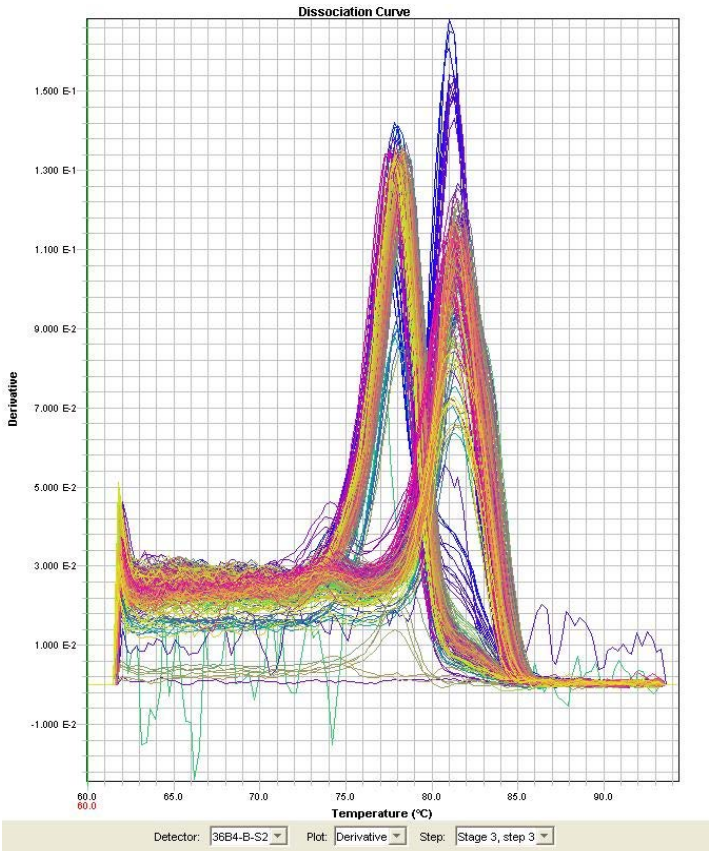


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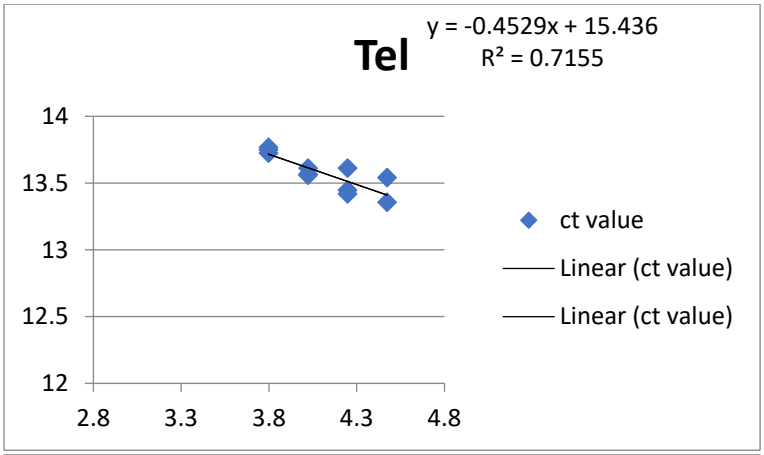
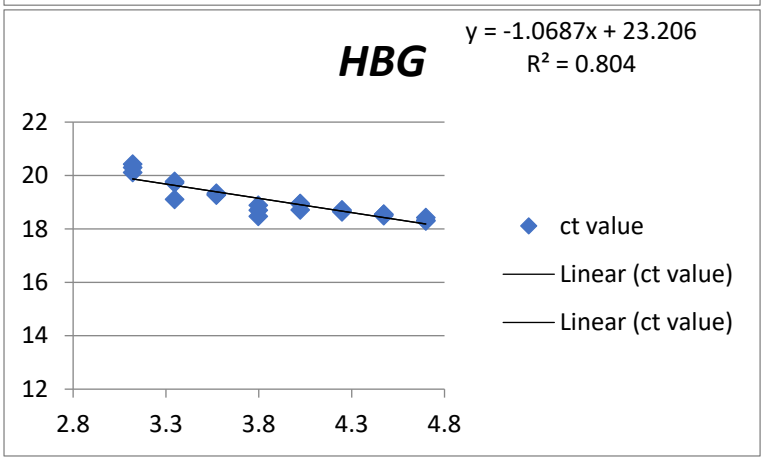
Plate 14





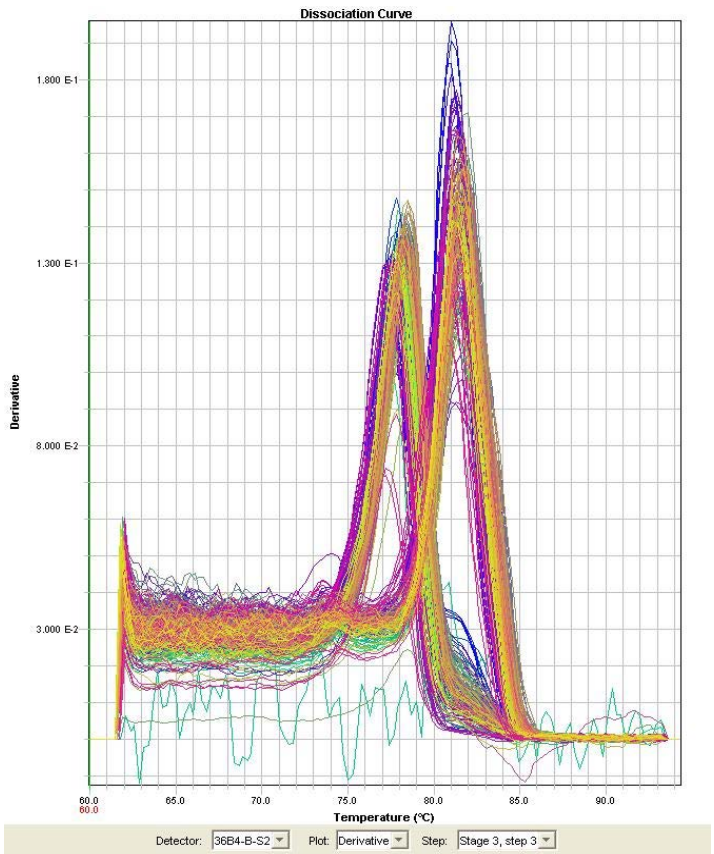


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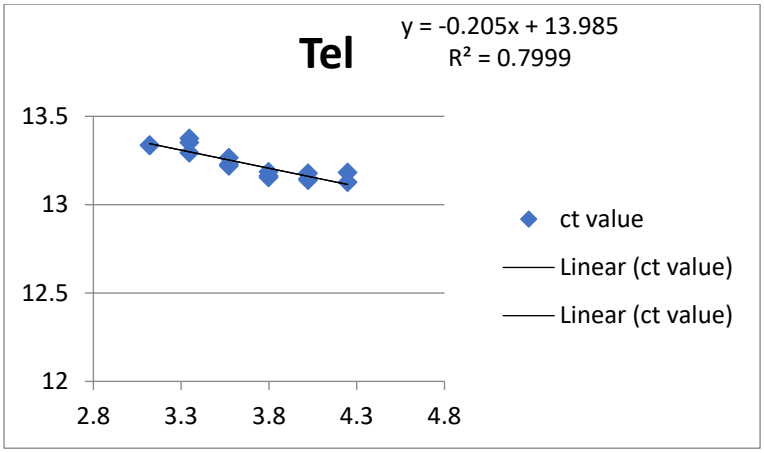
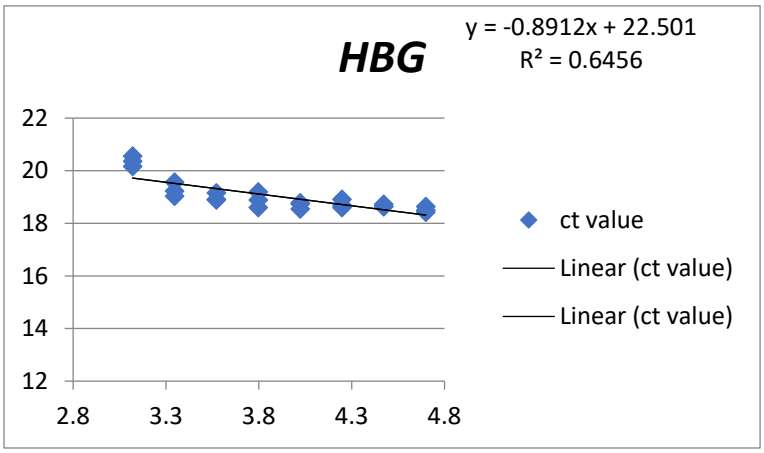
Plate 15





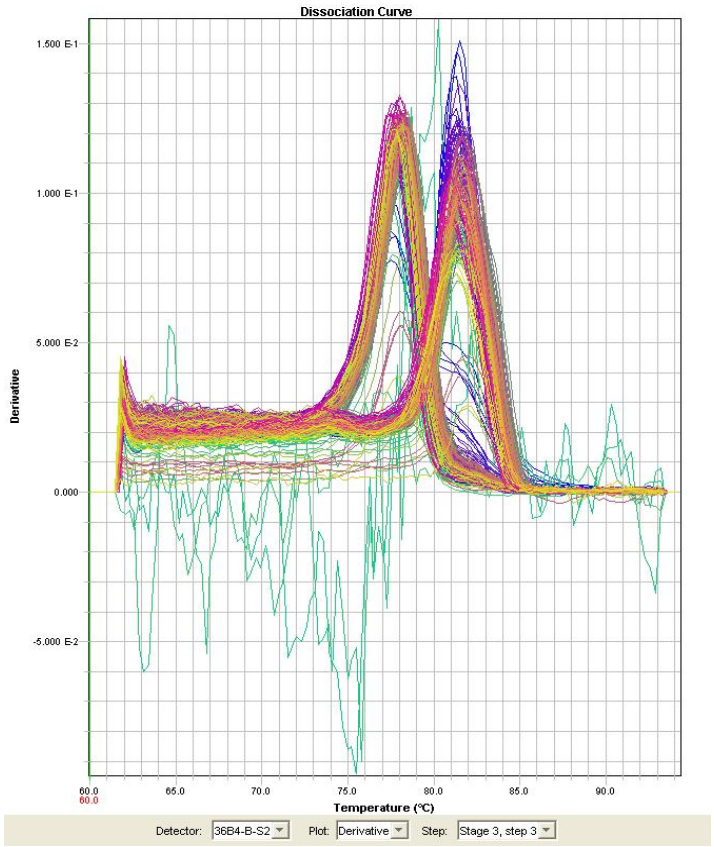


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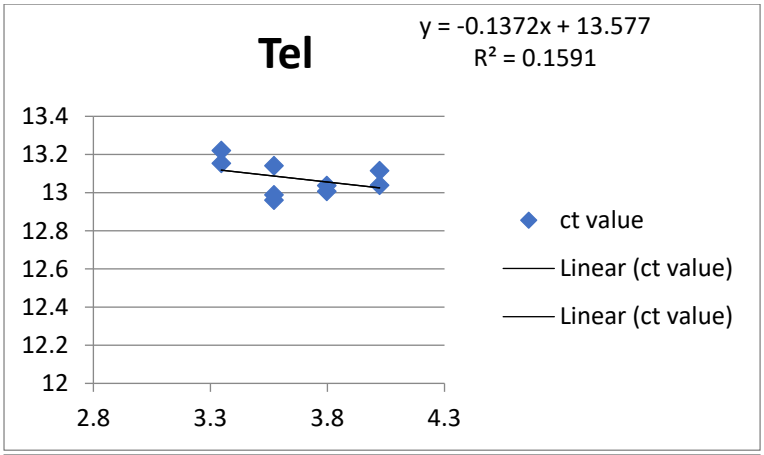
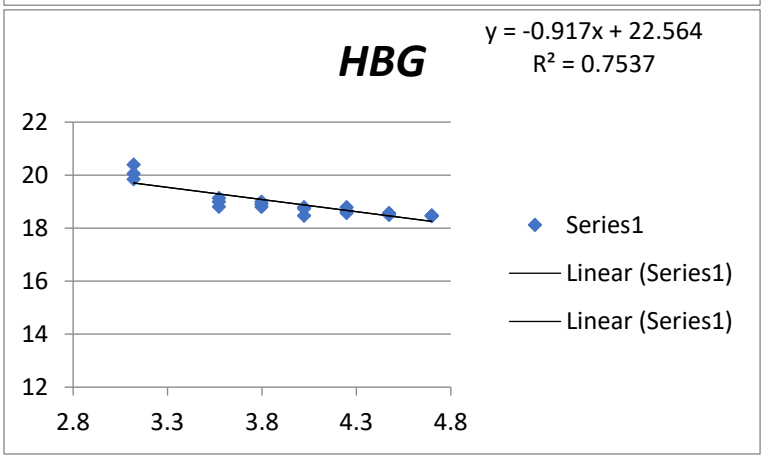
Plate 16





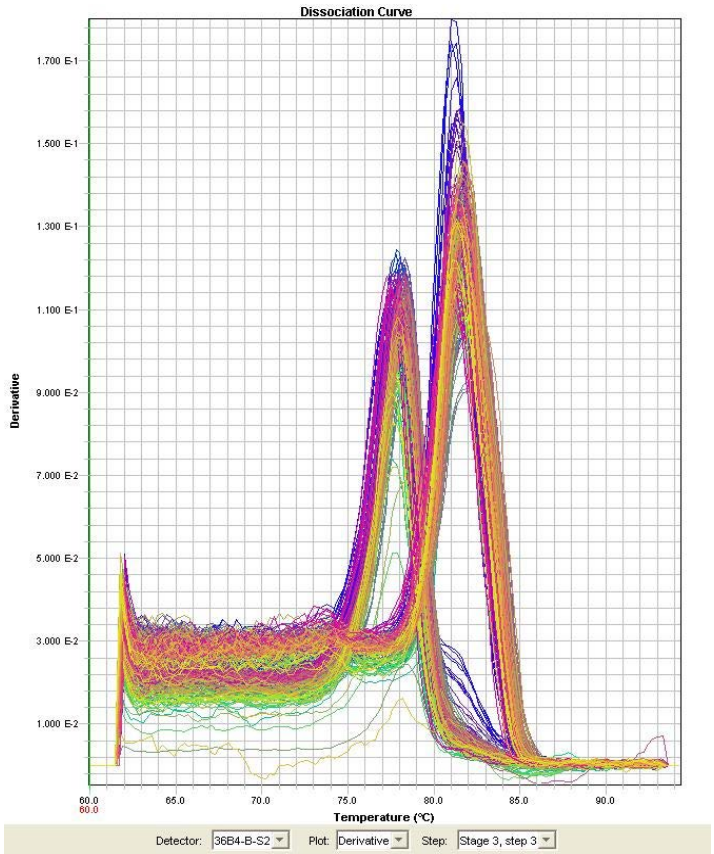


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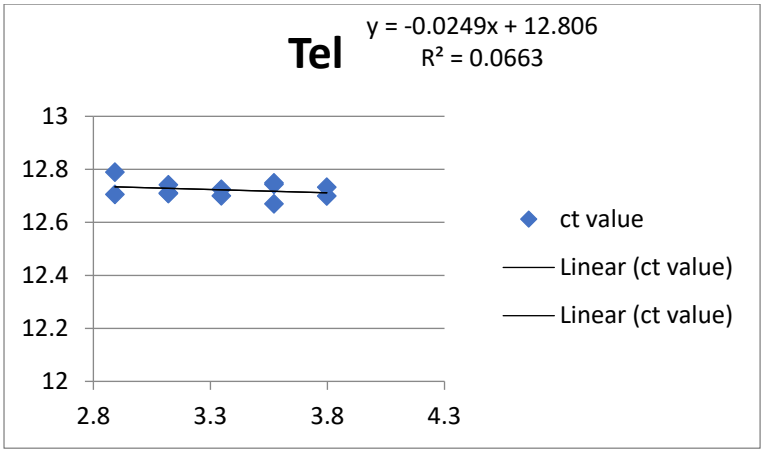
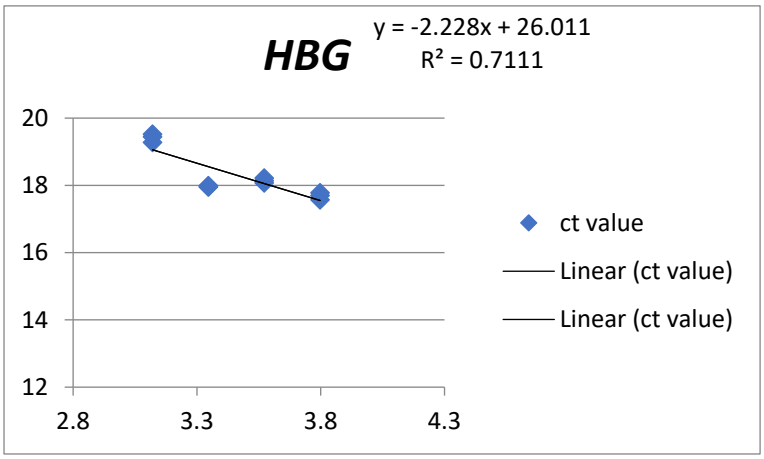
Plate 17





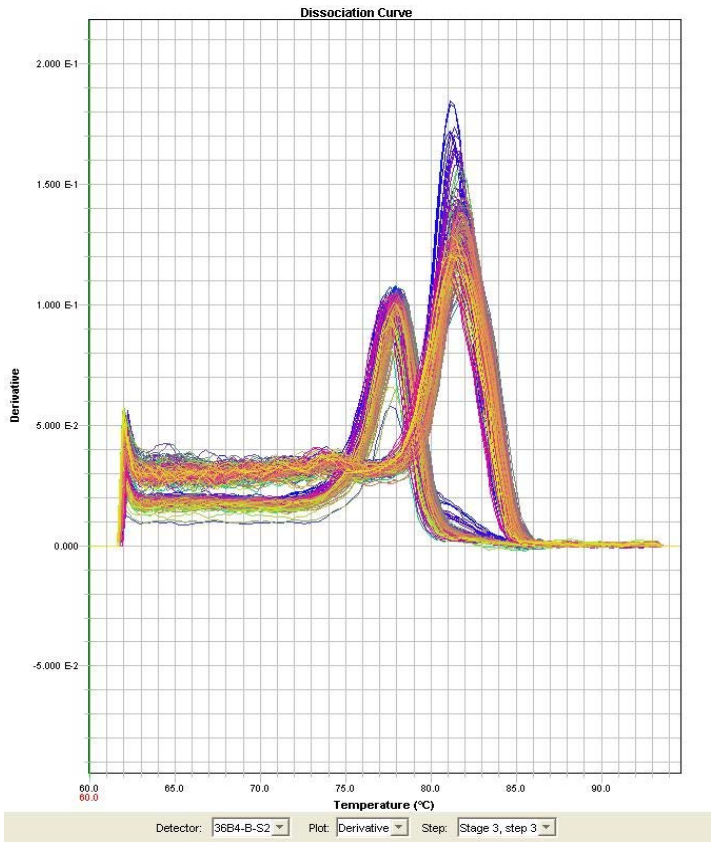


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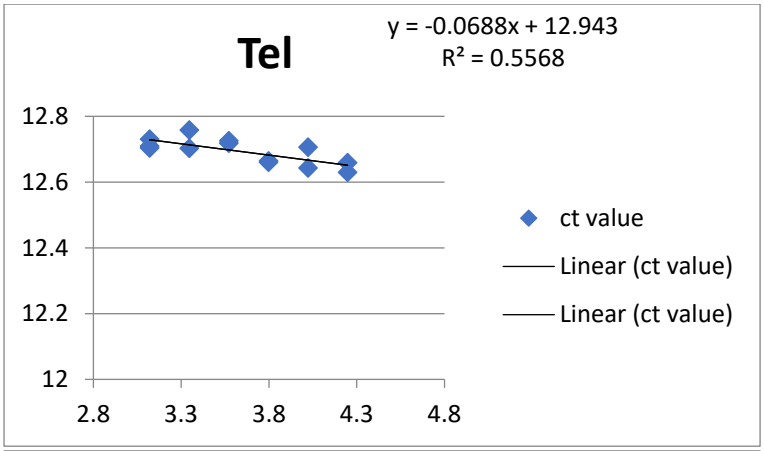
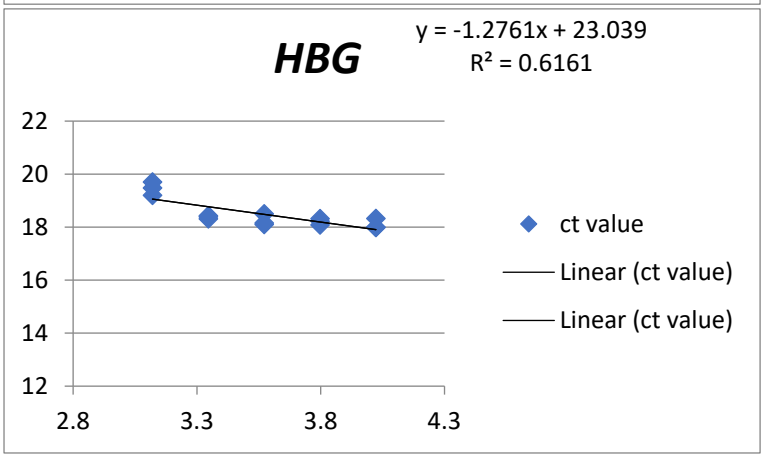
Plate 18





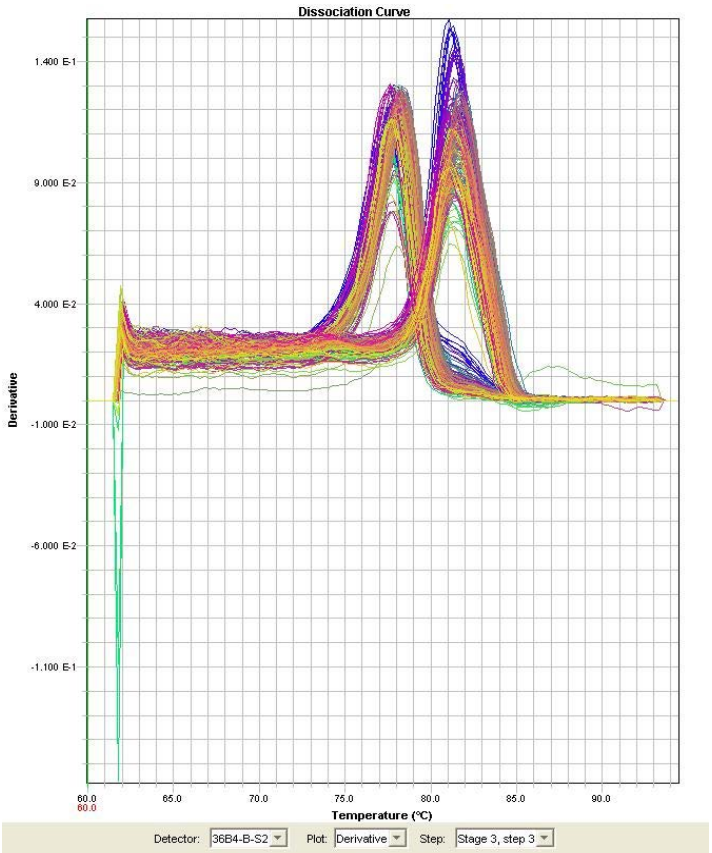


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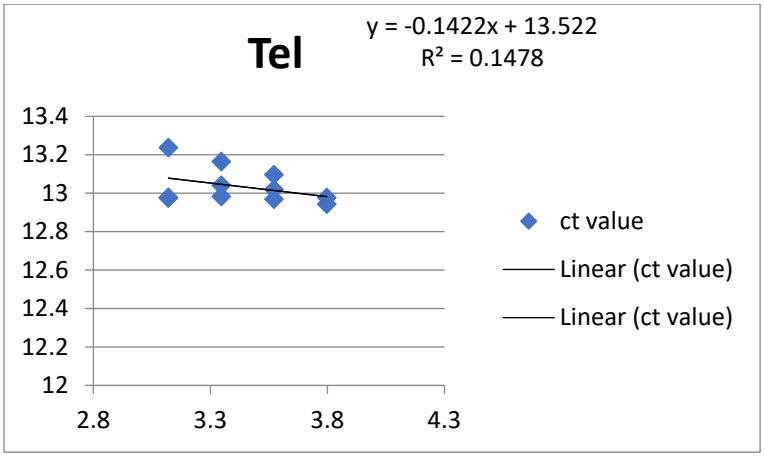
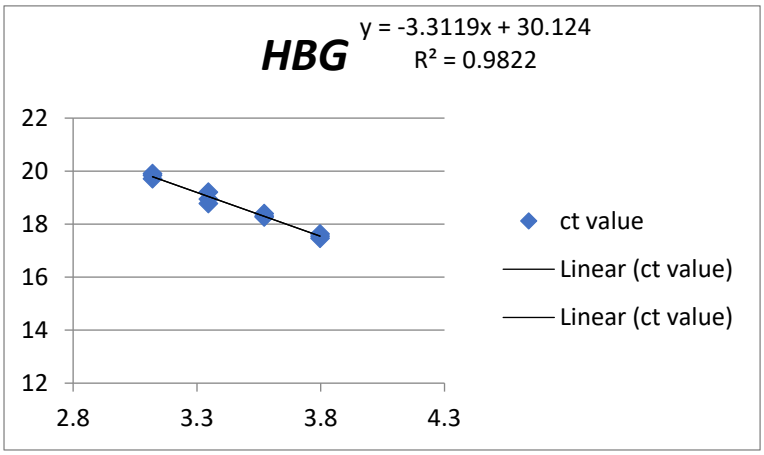
Plate 19





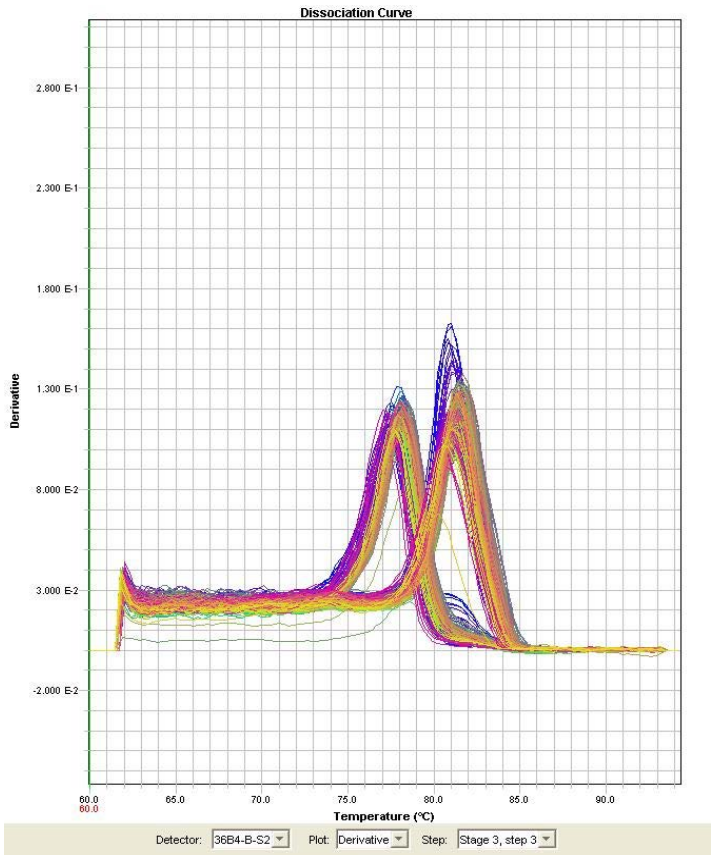


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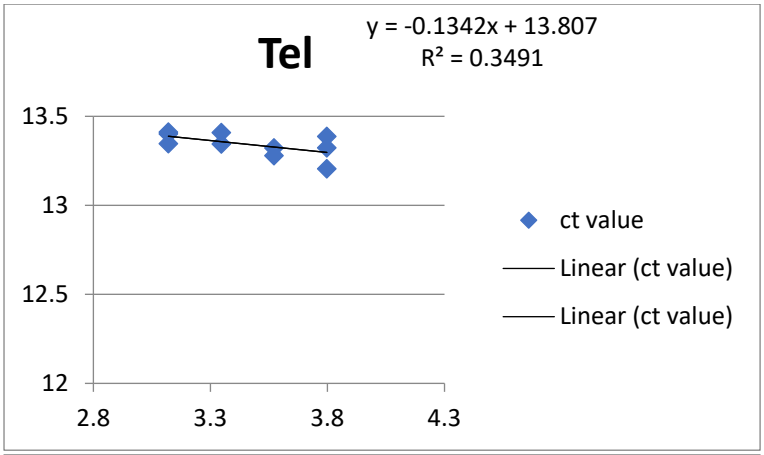
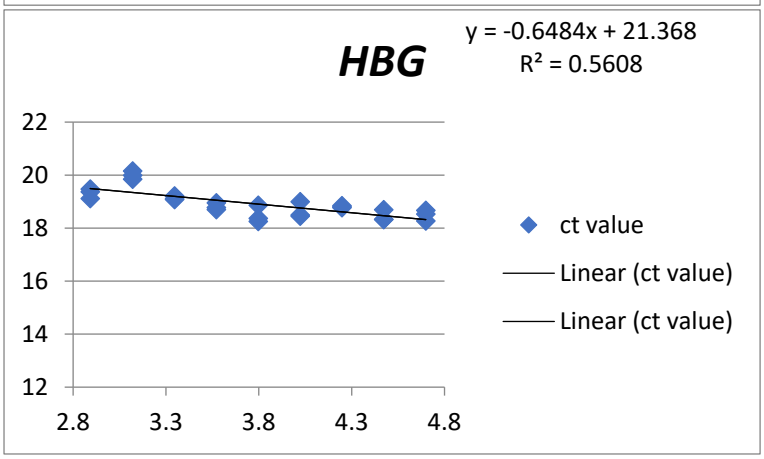
Plate 20





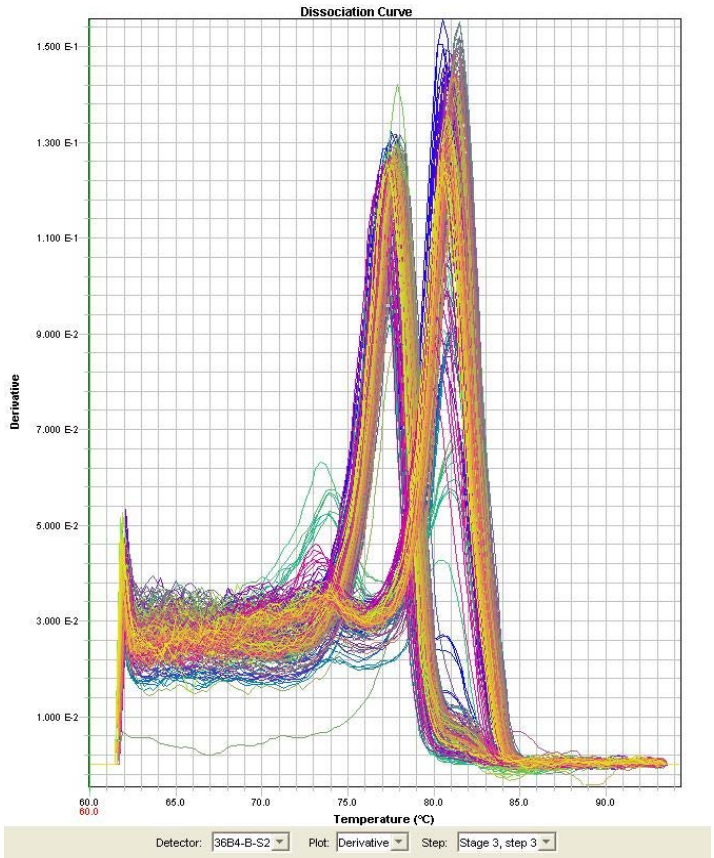


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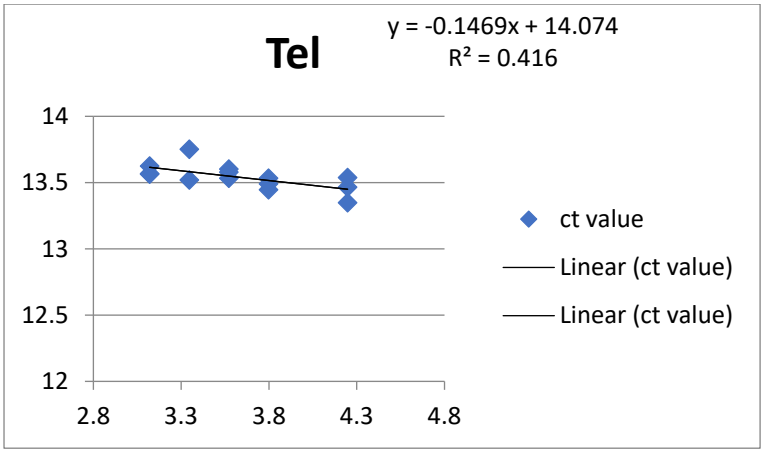
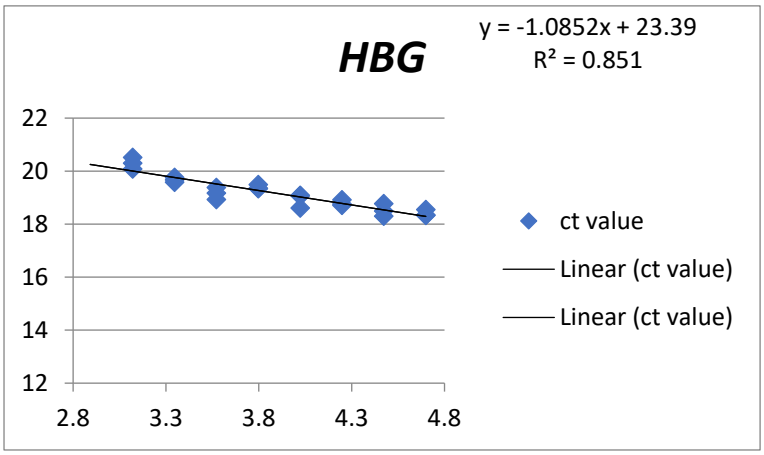
Plate 21





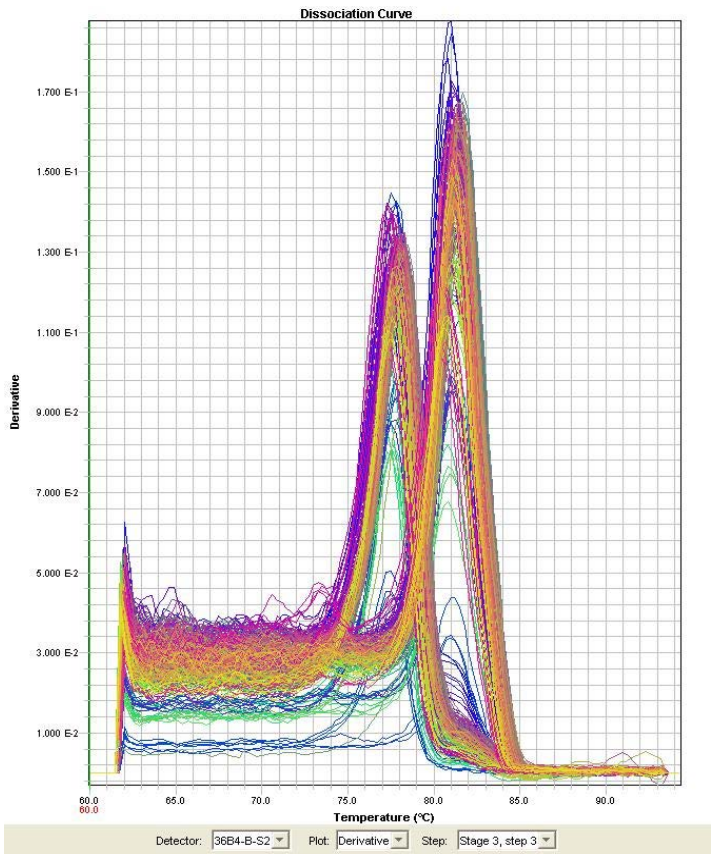


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Plate 22